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(*continued inside back of cover*)

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With 3 *text-figures*
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Structural polymorphism in *Drosophila subobscura* Coll. from various localities in Scotland

By G. R. KNIGHT

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(*Received* 10 *March* 1960)

INTRODUCTION

Philip, Rendel, Spurway & Haldane (1944) emphasize the almost universal presence of inversion loops in the salivary chromosomes of larvae obtained from wild parents of *Drosophila subobscura*. Structural rearrangements are found on all of the five long acrocentric elements of this species, in contrast to *D. pseudoobscura* where the bulk of chromosomal polymorphism is carried by the third chromosome (Dobzhansky, 1944). Because of this widespread polymorphism in *D. subobscura*, attempts have been made by many investigators to establish racial differentiation in the type and frequency of chromosomal arrangements.

Basden (1954) states that *D. subobscura* is the most widely and commonly distributed species of the genus throughout Scotland, and that its varied habitats extend over the whole of the mainland. It has been trapped at many sites inland and on the coast, as well as on some of the western isles, including the small island of Iona. This ubiquity of habitat in Scotland renders *D. subobscura* suitable, as it has been in other European countries and in Israel, for the study of potential geographic races within the species (Stumm-Zollinger & Goldschmidt, 1959).

Workers investigating salivary gland chromosomes of *D. subobscura* have adopted the Küsnacht stock as their standard. It was the first stock found structurally homozygous in all its chromosomes.

There has been in the past some diversity in the nomenclature used but the system devised by Mainx and his collaborators has now become accepted (Mainx *et al.*, 1953; Kunze-Mühl & Müller, 1958) and will be used here. The five long chromosomes are given the letters A, J, U, E and O respectively.

The structural arrangements have been summarized by Kunze-Mühl & Müller (1958), where a total of thirty-nine inversions are listed. In the present paper a new inversion from the north-central region of Scotland is recorded, and, added to the existing roll, makes, so far as is known, forty inversion types.

METHOD

Miss Mary M. Gunson, a research worker from Melbourne, Australia, while on a visit to the Institute of Animal Genetics, Edinburgh, made a preliminary study of samples of populations of *D. subobscura* from widely separated parts of Scotland.

The samples of flies were supplied by E. B. Basden (1954), who established in the laboratory a series of strains each from a separate locality. In every case the strain was derived from a single fertilized female captured in the wild. They were examined by Miss Gunson a few generations afterwards.

Miss Gunson (1952) merely recorded the occurrence of inversion loops on the separate chromosomes and roughly described their position. In our work about half of her slides were examined (the remainder having been accidentally lost) and the chromosome orders identified in both homozygotes and heterozygotes in accordance with the Mainx nomenclature. Slizynski's modified Feulgen technique was used to stain the salivary gland squashes (Gunson, 1952).

It was further arranged by the author to collect samples of two populations of *D. subobscura* within the county of Midlothian, Scotland. The sites chosen at Dalkeith and Heriot were ten miles apart. Both habitats were in mixed woodland and near to a river. The Heriot site was about 860 feet above sea-level amidst hill country south-south-east of Dalkeith.

The Dalkeith site in this experiment is at 160 feet above sea-level. It is also one mile north of the Dalkeith site in Gunson's investigation, with a built-up area intervening. Any differences in chromosomal frequencies found between samples trapped at those two sites will be interesting, remembering, however, that there was a period of several years between collections.

Traps as used by Basden (1954) were put out at the Dalkeith and Heriot sites in the month of October 1957. These were collected after 6 days' exposure during mild weather, and flies caught were segregated into species. Ultimate numbers of *D. subobscura* fertilized females were ten from Dalkeith and seven from Heriot. The resulting cultures used for investigation were therefore designated D10 and H7. From the larvae of the F_2 generation of these cultures a few diploid salivary preparations were made and male adults used for crossing with virgin ♀♀ of the standard Küsnacht stock. Twenty ♂♂ from each culture were used, each male being put into a vial along with one Küsnacht female. From the progeny of each pair-mating three larvae were sampled and permanent preparations made of salivary gland squashes (Slizynski 1952). Sixty haploid chromosome sets were examined from each site. The maximum numbers of chromosomes that could be tested from Dalkeith and Heriot were 40 and 28 respectively. In actual fact, however, these numbers would be less, due to the probability of some males contributing more than others to the progeny in the first two generations bred in the laboratory.

RESULTS

In Gunson's investigation the initial samples of *D. subobscura* from various localities in Scotland are small. Of the sixty-four individuals examined, thirty-seven were found to be structurally homozygous on all chromosomes. Table 1 lists the chromosome orders identified, and the sketch map (Fig. 1) shows the places of origin of those flies carrying particular orders.

Excluding standard, which was the order of all the A and J chromosomes, there

were identified in the other three elements only five different structural rearrangements, one of which, namely U_{1+2}, was found as a homozygote at several of the sites. Two exceptions occur. At Upper Loch Linnhe on the west-central coast, the U-chromosome in all the larvae except one was homozygous for standard. The odd larva carried the order $U_{1/1+2}$. At Stranraer, on the south-west coast, all larvae carried the rearrangement U_{1+2} except one which was standard.

The nature of these exceptions suggests that the two slides may have been accidentally interchanged in labelling. This has been assumed in the table and map. From the small western-island site of Iona, only a few chromosome sets

Table 1. *Chromosome orders present in* D. subobscura *sampled in Scotland in Gunson's investigation*

	Order					
Chromosome	Ullapool (north-west coast)	Stranraer (south-west coast)	Dalkeith (south-east)	Upper Loch Linnhe (west-central coast)	Drumnadrochit (north-central inland)	Iona (western island)
A	A_{ST}	A_{ST}	A_{ST}	A_{ST}	A_{ST}	A_{ST}
J	J_{ST}	J_{ST}	J_{ST}	J_{ST}	J_{ST}	J_{ST}
U	U_{1+2}	U_1 U_{1+2}	U_1 U_{1+2}	U_{ST}	U_{1+2}	U_{1+2}
E	E_{ST}	E_{ST}	E_{ST}	E_{ST}	E_{ST} $*E_{14}$	E_{ST}
O	O_{ST} O_{3+4}	O_{ST} O_{3+4} O_{3+4+8}	O_{ST}	O_{ST} O_{3+4}	O_{ST}	O_{ST}

* New inversion.

were available. The order was standard for all elements except in the U, which was homozygous for the inversion 1 + 2.

Gunson found only one inversion loop on the E-chromosome, at the Drumnadrochit locality. Its breakage points—proximal 67A, distal 70A/B on the new chart (Kunze-Mühl & Müller, 1958)—do not coincide with any already known inversions so far as can be ascertained by the author. It is therefore recorded here as new and named E_{14}. Fig. 2 shows the inversion loop formed with E_{ST}.

In the O element, the longest of *D. subobscura* salivary chromosomes, on which nineteen different gene arrangements are known, there were found the orders O_{ST}, O_{3+4} and O_{3+4+8}. The second was present at Ullapool, Stranraer and Upper Loch Linnhe. The Stranraer sample also carried the order O_{3+4+8}, which was not found elsewhere.

The 'dot' chromosome exhibited no discernible structural abnormality in any of the specimens.

Table 2 shows the results for *D. subobscura* trapped at Dalkeith and Heriot. The A-chromosome from both localities is entirely of the standard order, and this

chromosome at Dalkeith is the only one of the set to be entirely free from inversions. Heriot, in addition to the standard order of its sex chromosome, shows no heterozygosity on the E-chromosome, which, at Dalkeith, has the order E_{1+2} at a frequency of 8·3%. There is little difference at both sites between the frequencies of the orders J_1, U_{1+2} and O_{3+4}, though, in each case, the non-standard order is slightly more frequent at Dalkeith.

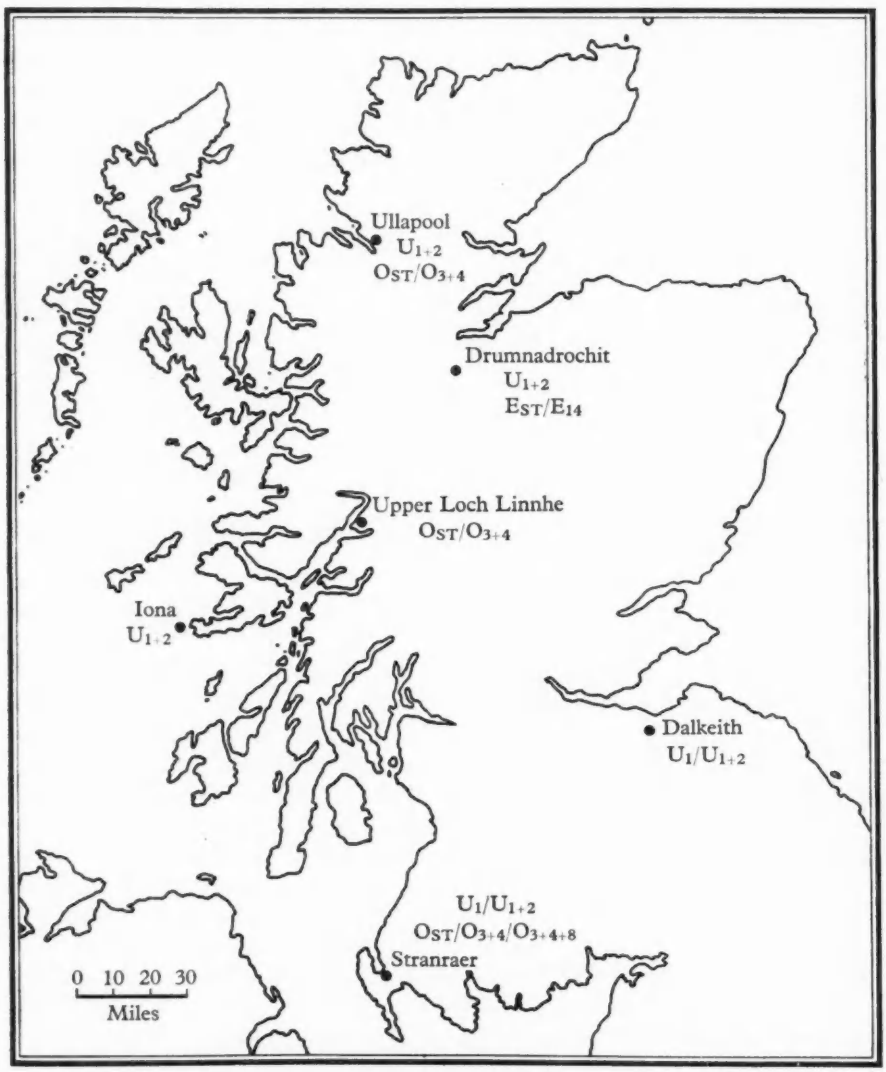


Fig. 1. *D. subobscura* chromosome orders found at various Scottish localities in Gunson's material. For the chromosomes not mentioned at a particular locality on the map only the standard order was found.

Table 2

A. *Analysis of haploid chromosome sets of* D. subobscura *showing frequency of orders found at Dalkeith and Heriot, based on 120 larval offspring of crosses: Küs. ST* ♀♀ × *D. & H.* ♂♂

Dalkeith (60 larvae sampled)				Heriot (60 larvae sampled)			
Chromosome	Order	No.	Frequency (%) of order	Chromosome	Order	No.	Frequency (%) of order
A	A_{ST}	60	100·0	A	A_{ST}	60	100·0
J	J_1	11	18·3	J	J_1	7	11·7
	J_{ST}	49	81·7		J_{ST}	53	88·3
U	U_1	—	—	U	U_1	11	18·3
	U_{1+2}	51	85·0		U_{1+2}	49	81·7
	U_{ST}	9	15·0		U_{ST}	—	—
E	E_{1+2}	5	8·3	E	E_{1+2}	—	—
	E_{ST}	55	91·7		E_{ST}	60	100·0
O	O_{3+4}	21	35·0	O	O_{3+4}	16	26·7
	O_{ST}	39	65·0		O_{ST}	44	73·3

B. *Analysis of larvae from females caught in the wild. Only the structural heterozygotes are detailed. Of the homozygotes, all chromosomes except the U were of the standard Küsnacht order, the latter having the order* U_{1+2}

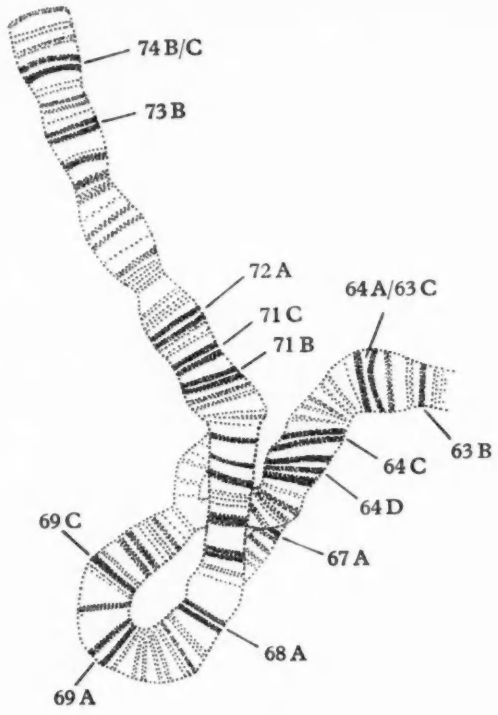
(6 larvae)	(6 larvae)
1. Homozygous for all chromosomes	1. U_1/U_{1+2}
2. E_{ST}/E_{1+2}	2. J_{ST}/J_1
3. O_{ST}/O_{3+4}	3. O_{ST}/O_{3+4}
4. Homozygous for all chromosomes	4. U_1/U_{1+2}
5. U_{ST}/U_{1+2}, O_{ST}/O_{3+4}	5. U_1/U_{1+2}, O_{ST}/O_{3+4}
6. U_{ST}/U_{1+2}, O_{ST}/O_{3+4}	6. J_{ST}/J_1, U_1/U_{1+2}, O_{ST}/O_{3+4}

There are then structural rearrangements on four of the chromosomes at Dalkeith and on three at Heriot. The two samples differ slightly in the U- and E-chromosomes. The U-chromosome shows heterozygosity at both locations, but U_1, present at Heriot, is replaced by U-standard at Dalkeith. Fig. 3 summarizes the inversion types at both localities.

DISCUSSION

Analyses of structural polymorphism in populations of *D. subobscura* so far sampled from Scotland are characterized by the simplicity of their inversions when compared with populations of this same species in Europe (Stumm-Zollinger, 1953) and Israel. Investigations from populations in the latter country have shown exceptionally complicated rearrangements (Goldschmidt, 1956, 1958).

The predominant rearrangement of the U-chromosome in Scotland is U_{1+2}. In



EST/E14

Fig. 2. Inversion loop of E_{14} formed with E standard.

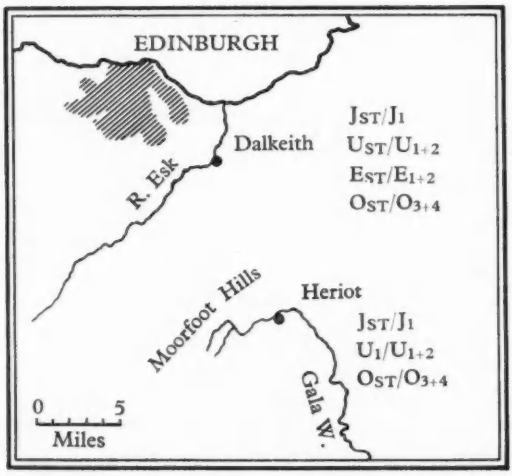


Fig. 3. Chromosome orders found at two adjacent localities in Midlothian, Scotland.

England that order and U_{ST} were found about equally distributed amongst a sample of flies from the New Forest (J. Maynard Smith, written communication). U_{1+2} rarely occurs by itself in Israel. There it is replaced by much more complex orders U_{1+2+7} and U_{1+2+3} (Goldschmidt, 1958). In France and Switzerland U_{1+2} is very common.

U_1 and U_{ST} were found at low frequency at Heriot and Dalkeith respectively.

The orders E_{1+2}, recorded only at the Heriot site, and O_{3+4}, present at most other Scottish localities, are found in similar frequencies in Europe.

The J_1 inversion which was present at Heriot and Dalkeith at low frequency has been recorded around 50% at European localities, and 29% from Israel (Goldschmidt, 1956).

Throughout this preliminary investigation of *D. subobscura* in Scotland, the outstanding feature has been the high proportion of homozygous types found. In particular, the seven larvae sampled from Iona showed a homozygous chromosome set, which, when compared with 'Kusnacht', was found to be standard in all its elements except the U. This chromosome carried the U_{1+2} inversion in homozygous order. It must be emphasized, however, that Gunson's samples were bred each from one fertilized female from the wild.

The method of measuring the degree of polymorphism of *D. subobscura* by Stumm-Zollinger & Goldschmidt (1959) in their European and Israel populations was that adopted by Carson (1955) using his 'index of free recombination' (I.F.R.) as a yardstick in his work with *D. robusta*. This criterion necessitates the estimate of the average lengths of euchromatin not involved by inversions. By this method Carson demonstrated successfully an increase from 65% in the centre of the distribution area of *D. robusta* to 85% in the marginal areas.

It is not possible to estimate accurately the index of free recombination, but from the frequencies of the inversions it would be expected to be around 92% for the Dalkeith population and 95% for Heriot. Stumm-Zollinger & Goldschmidt (1959) found an average of 85·0% for the continent of Europe. They found an average of only 4% of all females homozygous. The estimated figures for Dalkeith and Heriot are 24% and 32% respectively. In fact, in the samples discussed by Gunson which had been inbred for one generation after capture, the actual proportion of homozygotes was 58%.

Stumm-Zollinger & Goldschmidt (1959) found that the I.F.R. of populations of *D. subobscura* inhabiting various localities differed significantly. Whilst these authors agree that by using this standard a more delicate indicator of geographic differentiation is obtained, they are of the opinion that it by no means conveys the complete picture. They add that the most significant difference between European and Eastern populations lies in the number of elements involved in polymorphism. Differences in inversion frequencies and/or diversity of structural types suffice largely to characterize widely divergent populations. For instance, the chromosome order O_{3+4} is found to be common in Europe and rare or even absent in Israel, whilst the configuration J_{3+4} is common in Israel and scarce in Europe (Goldschmidt, 1958).

As Scotland and Israel can reasonably be assumed to be geographical marginal areas of *D. subobscura*, then analysis of chromosomal polymorphism from these two extreme localities might be expected to conform to the working hypothesis of Da Cunha & Dobzhansky (1954). Those authors, from the results of their investigations into structural polymorphism in *D. willistoni*, found a decrease in the degree of heterozygosity towards the margin of the species area. Though this is not the case according to results of analysis of *D. subobscura* from Israel, it does appear that the Scottish findings at present tend to agree with this theory.

This Scottish investigation is mainly exploratory, but results recorded here do give some indication of the diversity of inversion types to be found and warrant further research. Having this aim in view, samples of three *D. subobscura* populations were collected from regions separated from each other by two natural barriers, and analyses of their chromosome sets will be recorded later.

SUMMARY

1. Gunson's salivary chromosome preparations of *Drosophila subobscura* from widely separated sites in Scotland have been re-examined and inversions recorded according to the Mainx nomenclature.

2. Sixty-four diploid sets only were available. Of these, thirty-seven sets were found to be structurally homozygous on all chromosomes.

3. From Drumnadrochit in the north-central area of Scotland, the inversion found on the E-chromosome, so far as is known, has not previously been described. Its break-points have been noted, and the inversion is named E_{14}.

4. A strain of *D. subobscura* from the small western island of Iona was the only one found to be completely homozygous in the five long arms of the chromosome set.

5. Samples of *D. subobscura* from two closely related localities in Midlothian, Scotland, also have been examined. Results are based on the analysis of 120 haploid sets in hybrids between the local race and the standard Küsnacht stock.

6. A slight difference in type and frequency of inversions has been noted between the two populations. The inversion E_{1+2} was recorded from Dalkeith, but was absent at Heriot, while U_1, present at Heriot, was replaced by U_{ST} at Dalkeith.

7. The A-chromosome was structurally homozygous throughout.

8. Scottish samples of *D. subobscura* are characterized by their qualitative simplicity of polymorphism, the variety of inversion types being small. Chromosome orders analysed have been compared with those occurring in Western Europe and Israel.

It is a pleasure to thank Miss M. M. Gunson, of Melbourne, Australia, without whose salivary chromosome preparations of *Drosophila subobscura* the first part of this work could not have been carried out. To Dr Alan Robertson I wish to express my gratitude for helpful criticism and guidance throughout this investigation. I am indebted to Mr E. B. Basden for his assistance with the trapping and identification of the flies. And to Mr E. D. Roberts, my thanks are due for the preparation of the diagram and maps.

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Developmental sequence of chromosome number in a cytologically unstable Rubus hybrid

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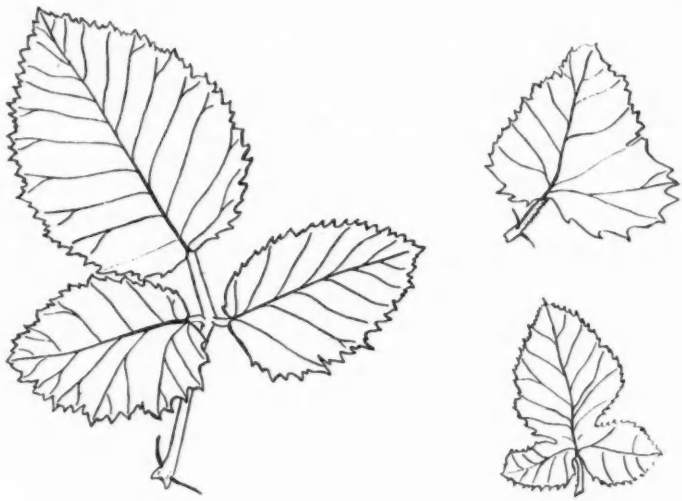
(*Received* 14 *May* 1960)

INTRODUCTION

One plant was observed to grow more slowly than the others in a population of thornless segregants in an F_2 from the cross of Merton Thornless (*R. craniensis*, $2n = 28$) with Himalaya Giant (*R. procerus*, $2n = 28$) (Plate I, fig. 1). This seedling was very stunted, with greatly shortened internodes, and with thickened and rugose leaves much smaller than those of normal sibs (Text-fig. 1). The plant was densely



Text-fig. 1. The leaves from (*left*) a normal stable tetraploid ($2n = 28$) thornless seedling ($1^2/59$) taken from the sixth node, and (*right*) leaves from the abnormal, cytologically unstable seedling of the same F_2, taken from the sixth and thirteenth nodes (Plant $1^1/59$).

covered with hairs, and very dark green. Even after twelve months it was approximately only 12 cm. high, compared with an average of 226 cm. for the normal sibs. At first it sent up one shoot only, but eventually it produced a short lateral shoot, although by then the normal sibs had produced three or four long shoots. The root system was also poorly developed, particularly in comparison with the normal vigorous rooting system of the sibs.

Table 1. *Sequential chromosome number variation in the cytologically unstable plant* 11/59

Sequential count	No. of cells with chromosome no.																							No. of cells	No. of chromosomes	Mean no. of chromosomes per cell	No. of roots examined
	9	12	19	21	23	24	25	28	29	30	31	32	33	34	35	36	37	38	39	40	46						
1	—	—	—	—	—	—	—	—	—	3	2	2	3	0	6	1	—	—	—	—	—			17	561	33·00	1
2	—	—	—	1	—	—	—	—	—	2	1	1	3	1	5	1	—	—	—	1	—			16	528	33·00	3
3	—	—	1	—	—	1	—	1	1	1	2	3	2	3	9	1	—	—	—	—	—			25	807	32·28	2
4	1	—	—	—	1	—	—	1	—	—	1	1	1	4	8	1	—	—	—	—	—			19	608	32·00	2
5	—	—	—	—	—	—	4	—	1	—	—	4	6	11	29	2	—	1	—	—	—			58	1954	33·69	3
6	—	1	—	—	—	—	—	1	—	—	—	1	3	1	43	—	—	1	—	—	1			52	1794	34·50	5
7	—	—	—	—	—	—	—	—	—	1	0	0	0	0	3	—	—	—	—	—	—			4	135	33·75	2
8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	42	1	—	—	—	—	—			43	1506	35·02	1
																										Mean:	
Total	1	1	1	1	1	1	4	3	2	7	6	12	18	20	145	7	0	2	0	1	1			234	7893	33·73	19

The period between the first and eighth count was 168 days.

Analysis of variance

Item	S.S.	N	M.S.	V.R.	P
Between counts	229·86	7	32·837	2·88	<·01
Within counts	2576·18	226	11·399		
Total	2806·04	233			

A detailed cytological analysis within this seedling has revealed great variation in chromosome number which also affects cell size. Britton & Hull (1956, 1957) have reported on mitotic instability in Rubus, but this paper provides the first sustained set of precise cytological observations made at intervals during the growth of a Rubus plant, not previously subjected either to vegetative propagation or to colchicine treatment.

METHODS

Root tips of the abnormal seedling ($1^1/59$) and of six normal sibs ($1^{2-7}/59$) growing in a cool glasshouse were pre-treated in 0·002 M solution of oxy-quinoline for 4 hours at 5 to 10° C. (cf. Tjio & Levan, 1950). The root tips were then fixed overnight in 1 : 3 acetic alcohol, and were hydrolysed in normal hydrochloric acid for 30 minutes at 58°–60° C. Then they were stained in Feulgen for 2 hours and squashed in carmine; the observations were made at × 1800 magnification. Later examinations were only on the abnormal seedling, but one normal plant was checked for stability at $2n = 28$ chromosomes at intervals. The records of the chromosome numbers are given in Table 1.

Some squashes were also made of stipules to see whether the instability was not necessarily confined only to the roots. Owing to the extreme density of the hairs, however, it was generally difficult to see good metaphase plates.

The measurements of cell size were obtained by making camera-lucida drawings at × 1200 on grid paper, and counting the number of squares (unit = 0·01 sq. inch) occupied by the cell.

OBSERVATIONS

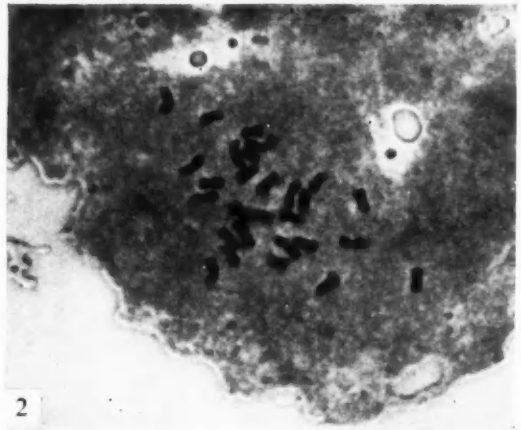
Variation in chromosome number

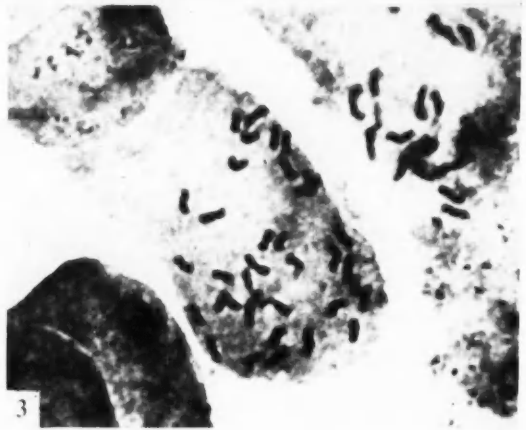
The somatic chromosome complement in a normal sib plant ($1^2/59$) is constant at $2n = 28$ (Plate II, fig. 1). The most frequent number in the abnormal plant $1^1/59$ is $2n = 35$ (Plate II, fig. 2); this plate shows no departure from the usual metaphase plate to be found in Rubus. The complete range of chromosome number within this plant is from 9 to 46. This means that cells lower than the diploid number ($2n = 14$) can occur. A sample of the range of chromosome numbers from 9 to 40 actually observed during the series of observations is given in Text-fig. 2. In Plate II, fig. 3, a metaphase plate with $2n = 12$ is illustrated, to show that there is no difference in the size of the chromosomes as compared with those in the $2n = 35$ cell. This means that reduction in chromosome number has not resulted from terminal fusion, as is known to occur in species like *Cardamine pratensis* (Lawrence, 1931).

The somatic chromosome count from a stipule cell ($2n = 39$) is shown in Plate II, fig. 4. Here the chromosomes look somewhat broader, but these had been pre-treated with colchicine instead of oxy-quinoline so any possible size difference of individual chromosomes might be attributable to the difference in technique.

Fig. 1. Two sibs from the F_2 of *R. craniensis* ($2n = 28$) × *R. procerus* ($2n = 28$). Photographed at 7 months.

Right: abnormal plant ($1^1/59$) with varying chromosome numbers.
Left: normal plant ($1^2/59$) constant with 28 chromosomes.





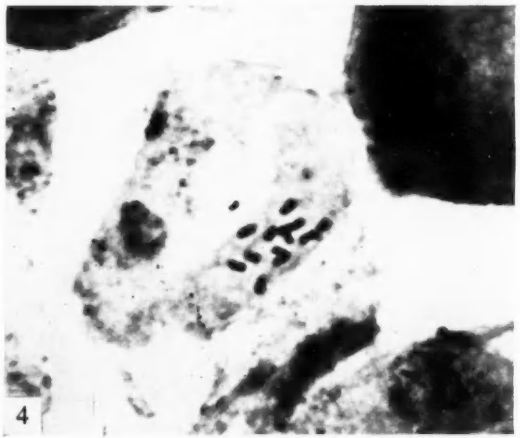


Fig. 2. Somatic chromosome number ($2n = 28$) from a root-tip of a normal sister seedling ($1^2/59$) pretreated with oxy-quinoline.

Figs. 3 and 4. Two chromosome counts ($2n = 35$ and 12 respectively) from root-tips of the abnormal hybrid $1^1/59$ pretreated with oxy-quinoline.

Fig. 5. Somatic chromosome count, from a stipule of the abnormal plant $1^1/59$ pre-treated with colchicine.

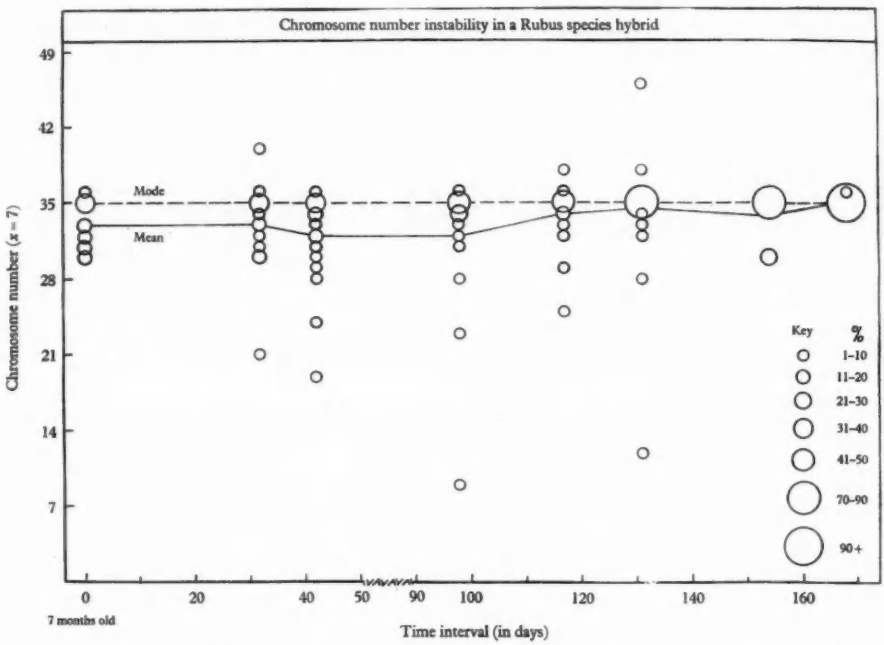
Text-fig. 2. Camera-lucida drawings of some of the varying chromosome numbers found in the root-tips of the unstable Rubus species hybrid ($1^1/59$).

A. $2n = 40$
B. $2n = 38$
C. $2n = 36$
D. $2n = 35$
E. $2n = 33$
F. $2n = 32$
G. $2n = 29$
H. $2n = 25$
I. $2n = 24$
J. $2n = 21$
K. $2n = 19$
L. $2n = 9$
M = Control ($1^2/59$), $2n = 28$

with oxy-quinoline.
Figs. 3 and 4. Two chromosome counts ($2n = 35$ and 12 respectively) from root-tips of the abnormal hybrid $1^1/59$ pretreated with oxy-quinoline.
Fig. 5. Somatic chromosome count, from a stipule of the abnormal plant $1^1/59$ pre-treated with colchicine.

Variation with age of plant

Text-fig. 3 shows the frequency of the various chromosome numbers, determined for a period of 168 days commencing when the plant was 7 months old. This diagram shows the mean chromosome number and also the mode, which is constant at $2n = 35$. During the period under observation the variation in chromosome number, although considerable, clearly is not related to the stage of plant growth. There is a suggestion, however, that there is less likelihood of very low chromosome



Text-fig. 3. The relative frequency of variation in somatic chromosome numbers of root-tip cells in the abnormal seedling. Observations were commenced at 7 months and continued for 168 days.

numbers occurring in later observations, and a greater likelihood of the occurrence of chromosome numbers higher than 35. Throughout the series of observations the mean chromosome number is lower than the mode, i.e. one is more likely to find cells with numbers lower than the pentaploid value of 35. A further interesting feature is that the relative frequency of the $2n = 35$ cells increases with age; this might be a likely consequence of an immediate effect on the cells from actual chromosome number. Throughout the series, the frequency of orthoploid cells with 7 as the basic number does not otherwise increase. Nor is there a balance towards cells with complete chromosome sets of $2n = 28$, which is the number for most species of Rubus; and at no instance during the period of the investigation was the somatic number only $2n = 35$.

An analysis of variance (Table 1) shows that there is a highly significant difference in chromosome numbers of the various counts. On two occasions counts were made on three roots, and five roots on one occasion. An analysis was carried out to determine whether the five roots differed in their chromosome numbers at any one time. This showed no significant difference between roots. The plant $1^1/59$ is, therefore, unstable with regard to the sequential date when roots are examined, but the roots themselves are generally behaving in a similar fashion.

Satellites

Satellites are a feature of Rubus. A pair of satellited chromosomes were observed in cells of the normal plants with 28 chromosomes; but in the abnormal plant one cell of 24 chromosomes had only one satellite, whereas two cells with 35 chromosomes had two and three satellites respectively (Table 2). This suggests that it is not always the same chromosomes that are being eliminated from one cell to another at mitosis.

Table 2. *Variation of satellite number in Rubus*

Species	Ploidy	Range of satellites per cell
R. tomentosus	$2x$	2 (15)
R. ulmifolius alba	$2x$	1 (5); 2 (25)
R. ulmifolius	$2x$	1 (7); 2 (75)
R. ulmifolius inermis	$2x$	2 (23)
R. tomentosus ×	$3x$	1 (14); 2 (12)
R. ulmifolius ×	$3x$	1 (1); 2 (8)
R. caesius	$4x$	2 (15); 3 (3); 4 (2)
$1^1/59$	$2n=24$	1 (1)
	$2n=33$	2 (1)
	$2n=35$	1 (1); 2 (7); 3 (4)
$1^{2-7}/59$	$2n=28$	2

Numbers in brackets equal cells with the corresponding nos. of satellites.

There are always two satellites per cell in the primary diploid ($2n=14$) species *R. tomentosus*, but in various normal tetraploids so far examined by us four satellites have not always been observed. This difference might result from technical difficulties in observation, e.g. from a satellite folding under a chromosome, or from amphiplasty as suggested by Vaarama (1953) and by Heslop-Harrison (1953). There is generally an increase in satellite number with increase in chromosome number, but the relationship is not absolute.

THE ORIGIN OF CHROMOSOME NUMBER VARIATION

The absence of virus

Tests were made for virus infection, as Caldwell (1952) has suggested that viruses might have wider effects on nuclear division than have been supposed, and as Rubi

generally are susceptible to virus infection. Sub-terminal leaves of the abnormal seedling ($1^1/59$) and a normal tetraploid sib ($1^2/59$) were removed, ground with alumina and rubbed on *Chenopodium alba*. These inoculation tests gave no indication of the presence of any of the five sap transmissible viruses. The cause for the instability must be sought elsewhere.

Lagging chromosomes

Most of the observations have been made on pre-treated root-tip cells, which would lessen the chances of seeing the mechanism responsible for maintaining the chromosome number variation. Only in one instance was it possible to make counts on two adjacent cells. One cell had 35 chromosomes and the other had 31 chromosomes; but this need not necessarily mean that both were the products of the same mitosis. Some observations were made later on root-tips without pre-treatment, but by then the chromosome number was beginning to stabilize at 35 and no abnormalities of mitosis, especially regarding spindle formation, could be seen. But one or two dumb-bell shaped nuclei were observed, and these may be comparable to the lobed nuclei observed by Britton & Hull (1957) in their mitotically unstable Rubus plants.

Instability of the egg cell

Plant $1^1/59$ is not like a true pentaploid Rubus plant with $2n = 35$ that has become unstable. Other Rubi of this ploidy are like the Mertonberry, a raspberry-blackberry hybrid (Crane & Thomas, 1949), which is cytologically stable and has large leaves and is vigorous and normal in appearance; it is also highly fertile. Neither is the instability a necessary consequence of species hybridity, for others also were normal and triploid seedlings from *R. rusticanus* ($2x$) × *R. thyrsiger* ($4x$) have remained vigorous and vegetatively stable, although with sterile flowers, for more than 25 years. Hence as the abnormal behaviour of plant $1^1/59$ is not readily attributable to either its being an odd-numbered polyploid or to its being a species hybrid, it must result from an intrinsic chromosome number instability leading to a general state of hormonal unbalance. It would seem that the instability first arose in the egg cell, as the pollen of Rubus is a sensitive reactor to chromosome unbalance (Gustafsson, 1943).

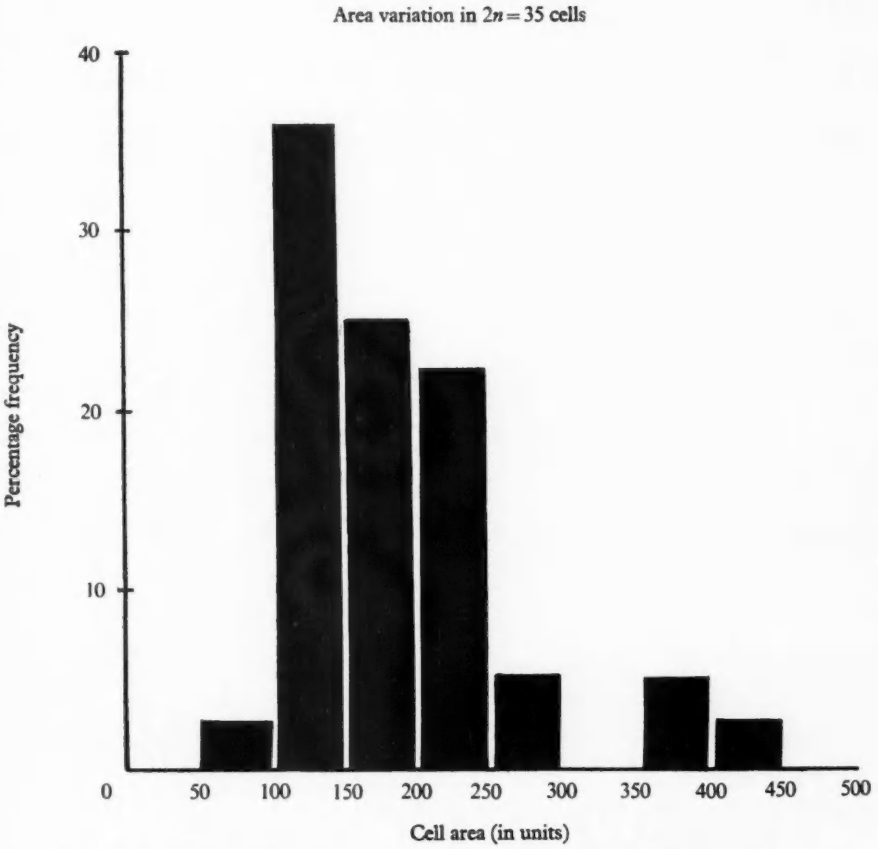
CHROMOSOME NUMBER AND CELL SIZE

Size variation within 2n = 35 cells

Although variation in cell size may be influenced by the technique of squashing not being always at the same pressure in different cells, there is the possibility that a variation in the chromosome karyotype itself may be the basis for the wide range of variation in the distribution for cell area, measured in units (Text-fig. 4). Here the histograms show a skewed distribution, with few cells smaller (viz. 50–100 units) than the most frequent class (101–150 units). Yet there is considerable extension of the skewness, so that there are to be found some cells falling into the

class group (401–450). It is recognized that these are the pooled measurements of cells from various roots, and there might have been some variation between roots. Furthermore, it is known that the volume of cells in the primordia and internodes of *Lupinus albus* is dependent on position (Sunderland, Heyes & Brown, 1956).

The constitution of the chromosomes in the $2n=35$ cells recorded at the commencement of observations need not necessarily have been the same as the $2n=35$



Text-fig. 4. Variation in area of a sample of cells in plant $1^1/59$ containing 35 chromosomes.

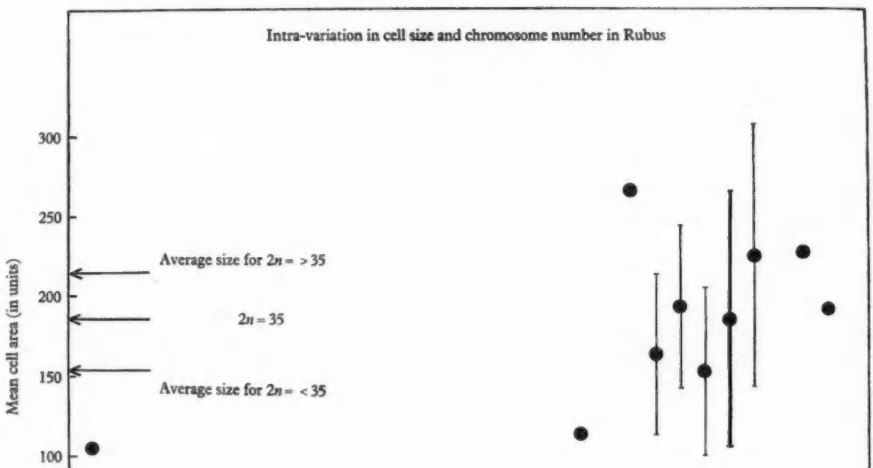
cells measured later, for the same chromosomes may be increased or decreased numerically through a series of unstable mitoses, and so the value '$2n=35$' itself becomes a misleading guide to chromosome constitution. This is even more of a possibility not only when taken in conjunction with the reports of Sharma & Sharma (1957) on mitotic changes in chromosomal karyotypes of various species, but also because the genes so far tested (7 out of the 17 known) in *Rubus idaeus* have been assigned to two linkage groups only. This might indicate that of the

basic 7 chromosomes, some are almost genetically inert, at least for oligogenes. Hence, if this were so, any of these chromosomes could be interchanged during successive somatic mitoses, without particular detriment to the plants, other than for biometrical effects which ensue from changes in the polygenic system. Their interplay may also effect vegetative segregation, which is a widely recognized feature of Rubus, e.g. having been observed several times in the *S/s* and the *H/h* genes, and it may also partly explain the variation in the vegetative parts of the plant $1^1/59$.

The occurrence of Rubus chromosomes as a cohort of 7 is essential to give balance to the polyploid series, and to the production of fertile pollen, necessary for the maintenance of the species, whether they are sexual or apomictic (Haskell, 1960*a*). Thus '$2n=35$' cells in this unstable plant may or may not all have the same genomic constitution.

Size variation in cells of a chromosome number series

There is a correlation between cell area and actual chromosome number within the root-tips of $r=+0{\cdot}65$, which is significant at the 5% level. The average size for the $2n=>35$ cells is 214 units, and for $2n=<35$ cells it is 154 units, and the



Text-fig. 5. Mean areas of cells from plant $1^1/59$ plotted against the number of chromosomes within those cells. Twice the standard deviation is shown in the larger samples.

mean of the $2n = 35$ cells is 186 units. Thus clearly the size of the cells is associated with chromosome number. It would appear, too, that euploid cells with the strict multiple of the basic number are not necessarily intrinsically larger in size than aneuploid cells, for the mean of the $2n = 35$ cells fits in with the general regression (Text-fig. 5). Thus cell size, at least in Rubus, appears to be more influenced by actual chromosome number than by a euploid constitution.

The steady increase in cell area with increase in chromosome number also suggests that variation in pressure during squashing does not have the influence that might have been expected. Hence it might not have so greatly affected the variation in size of the $2n = 35$ cells (Text-fig. 4), making this due more likely to the other suggested causes. The changes in variation within the cells carrying a particular chromosome number are seen from Table 3. The variation is found to

Table 3. *Internal variation in cell area for cells of different chromosome number*

Chromosome number	Departure from $2n = 35$	Mean area (in units)	Coefficient of variation
36	+1	226·1 ± 81·8	36·18
35	0	186·5 ± 78·4	42·04
34	−1	152·6 ± 52·2	34·21
33	−2	193·1 ± 50·8	26·31
32	−3	163·8 ± 49·5	30·22

be greatest in the $2n = 35$ cells, and there is some decrease in the coefficient of variation (C) with the addition or subtraction of 1, 2 or 3 chromosomes. Variation in cell area is not, therefore, most stable at the euploid level.

DISCUSSION

The basic chromosome number in the genus Rubus is $x = 7$, and polyploidy, especially tetraploidy, predominates in most true blackberries (Eubatae). Exceptions from the euploid series have been recorded: Crane (1940) had a dwarf monosomic ($2n = 27$) seedling following the selfing of tetraploid *R. thyrsiger*. Gustafsson (1943) found aneuploidy in *R. pyramidalis* Kalt. with $2n = 26$–28.

Chromosome number is more flexible in the Corylifolii (i.e. *R. caesius* and its hybrids), both in degree of ploidy and in occurrence of aneuploid numbers. Heslop-Harrison (1953) considered aneuploidy as rare in the subgenus Eubatus wild in Britain, and in several other Rubus subgenera. Among seedlings of North American polyploid Rubi, Einset (1951) found several aneuploids, but none were unstable. One small rosette plant, a $2n/4n$ mixoploid chimera, was found by Haskell (1960*b*) among diploid progenies of raspberry (Idaeobatus) from seeds harvested wild in Britain; it produced multiple growing points that failed to elongate and flower.

Therefore instability, whether cytological and/or morphological, is not necessarily an adjunct of either aneuploidy or of tetraploidy within diploid tissue.

However, in some vegetatively reproducing plants Sharma & Sharma (1957) have reported abundant varying chromosome complements in root tips, contrasting with sexually reproducing dictyledons.

Hegwood & Hough (1958) found a mosaic of chromosome numbers in apples with modes either at the diploid or triploid values, apparently due to abnormalities in mitosis. The tendency seemed to be heritable. Cells with double chromosome numbers scattered in normal root-tips occur in *Soldanella* spp.; Satczek (1951) attributed this to low temperatures following snowfall. The earlier observations, providing more of the lower chromosome numbers in plant $1^1/59$, were from roots growing at lower temperatures, very short days, and weak light intensity; but this applied also to the tetraploids. This conflicts with the hypothesis that tetraploid Rubi of Europe may have spread and replaced primary diploids following retreat of the ice front, i.e. following subjection of the diploids to low temperatures (cf. Gustafsson, 1943). Hull & Britton (1958) found in cuttings of a Boysen × Eldorado plant some cells with both high (e.g. 35–60) and low chromosome numbers. Re-examination in the following spring gave only low chromosome numbers (e.g. 20–35). This may represent differential growth rates of cells with varying chromosome numbers at different seasons of the year, or that lower temperatures during growth of a Rubus plant may reduce chromosome numbers, particularly in mitotically unstable individuals.

Various aneuploid complements occur in isolated cells of the orchid *Paphiopedilum wardii* root tips, although only three types might be multiplied of the twenty kinds of chromosomes (Duncan, 1945). They occur through a process resembling that for polysomaty, but not all chromosomes are replicated. Aneusomaty may be acting in seedling $1^1/59$, but owing to the difficulty in identifying individual Rubus chromosomes this has not been verified. The phenomenon is different from true endomitosis, not uncommon in Rubus, as illustrated by diploid *R. idaeus* which produces tetraploid sports from diploid shoots.

Spindle abnormalities were responsible for the instability in root tip cells and P.M.C.s of *Hymenocallis calathinum* (Amaryllidaceae) (Snoad, 1955). Chromosome number mosaicism also occurs in plants with stable root tips, e.g. Sachs (1952) found mosaicism in P.M.C.s of amphiploids involving Triticum, Aegilops and Agropyron, and spindle abnormalities might have occurred late in development, i.e. during premeiotic mitosis. Britton & Hull (1956) studied mitotic instabilities of six plants from Boysen × Eldorado and Young × Eldorado in Maryland (latitude 38° 55′), but their chromosome counts could only be ascertained with an error of ± 2 chromosomes. Accurate records have been made from clear plates only in the present observations. Although taken at latitude 56° 28′, i.e. almost 18 degrees further north of Maryland, which involves wide differences in daylength and light intensity, the same general pattern of instability occurs, providing that the plant under examination is itself unstable.

Besides the suggestion that the egg is a primary cause of instability, there is also some evidence ruling out an apomictic mechanism as responsible. Thus the F_1 plant that produced $1^1/59$ was vigorous and thorned, and might have produced

some progeny apomictically, for the parental *R. thrysiger* is a tetraploid apomict. Yet there is no indication that the instability arose from an apomictic egg, for the F_1 parent was heterozygous for the thorned (*S*) *v.* thornless (*s*) gene, and as plant $1^1/59$ is thornless it must have arisen sexually to be homozygous recessive, even allowing for the diplorous mechanism possibly involving a modified meiosis and recombination.

Morphological mosaicism occurs in polygenomic Gossypium hybrids, some characteristics being more frequent among hexaploid ($2n=78$) combinations than corresponding tetraploids (Menzel & Brown, 1952). The mosaicism was absent in aneuploid species with one or two extra chromosomes, and does not appear to result from interaction of specific incompatible genomes, but occurs in all hexaploid hybrids. Thus one hexaploid hybrid combining three genomes had a sector with altered chromosome number, and also some buds with $2n=65\pm$; and, less frequently, 2–6 extra chromosomes. This parallels the situation in plant $1^1/59$, where eighty-eight counts of cells gave the somatic number less than 35, and eleven counts gave more than 35 chromosomes (Table 2). Yet the polygenomic history of this plant, involving *R. ulmifolius*, *R. thyrsiger* and *R. procerus*, cannot itself be the prime cause of the instability, as its sibs are vigorous, constant tetraploids.

If the plant consisted of $2x$, $3x$, $4x$, etc., cells, it might merely be a polyploid mixo-chimera; but the behaviour is more complex, as the observed numbers do not correspond to a simple polyploid series. As the most frequent chromosome number is 35, one might expect cells with 34 or 36 chromosomes due to occasional lagging of a chromosome at anaphase; but these numbers do not preponderate. Furthermore, there is no corresponding chromosome number to balance with the same frequency the particular chromosome number to make up to 35 ($=5x$). Nor do we know whether mitosis takes place at the same rate in the cells with different chromosome numbers. There is no extra frequency of cells other than those with 35 chromosomes carrying the orthoploid series of the basic number $x=7$, viz. 14, 21, 28 and 42.

Although attempts were made to obtain chromosome counts from leaves and buds, the rugose nature and dense hairiness prevented this, but one plate was clearly countable from a stipule. This cell had 39 chromosomes (Plate II, fig. 4). In this respect Berger & Witkus (1954) have shown in *Xanthisma texanum* ($2n=8$) that only plants with shoots having supernumerary chromosomes produced occasional root cells with 9 or 10 chromosomes, but they never constituted more than 2% of the dividing cells. Elimination of the supernumeraries in the cells of Xanthisma root tissue during early differentiation results from lagging of the supernumeraries at mitosis (Berger *et al.*, 1955).

In a literature survey on cell size and polyploidy, Noggle (1946) suggested that 'chromatin : cytoplasm' ratios are different between diploid and tetraploid cells within various species; but they depended on the stage of development of the organs carrying the cells. The ratios for root tip cells in plant $1^1/59$ carrying the series of aneuploid chromosome numbers are given in Table 4. Here each ratio is

Table 4. *The* $\frac{\text{'Chromatin'}}{\text{cytoplasm}}$ *ratio in cells with increasing chromosome numbers*

Chromosome no.	Ratio
(9	11·78)
(19	3·42)
29	3·91
(31	8·97)
32	5·12
33	5·85
34	4·49
35	5·32
36	6·28
(38	6·02)
(39	4·95)
Mean	6·01

Figures in brackets are for single counts only.

assessed from the value of $\frac{\text{mean cell area}}{\text{chromosome number}}$. There is no trend in the series between chromosome number and the chromatin : cytoplasm ratio, so this constancy within similar tissue indicates evidence of a balance between cell size and chromosome number, and this balance leading to stability between the chromatin and the cytoplasm is predetermined.

On the other hand, there is correlation between cell area and chromosome number (Text-fig. 5). In liver and kidney cells of rats (Harrison, 1951) there is a direct relationship between the numbers of chromosomes within the cells and the amount of DNA per nucleus. It is not, therefore, unfeasible to suggest that in the chromosome mosaic root tissue of plant $1^1/59$, cell size and the amount of DNA are correlated, and that the quantity of DNA decides the ultimate size of the particular cell after cell-wall formation. Because the chromosomes of Rubus are alike in size, this produces a significant correlation in the aneuploid series, for the addition of single chromosomes is tantamount to adding a proportional quantity of DNA within cells of the same tissue and growing under similar environments.

The discovery by several workers that mitotic instability occurs in Rubus plants of widely different sources, e.g. from British and North American origin, indicates that this is more widely spread than previously supposed. Interesting material is now available to study how important is this form of somatic variation in the life-cycle in Rubus, where vegetative reproduction is prominent. That there is a mechanism affording somatic variation in Rubus is in line with earlier knowledge of the versatile breeding system of the genus. It may also help to explain why some varieties of Rubus in cultivation have changed ploidy since chromosome counts were first made. Thus Crane & Darlington (1927) originally gave the chromosome number of Himalaya Giant (*R. procerus*) as $2n = 49$, but all recent counts have been consistent at $2n = 28$; the Mahdiberry, previously a semi-sterile triploid with $2n = 21$, is now a fertile pentaploid with $2n = 35$ (Crane & Thomas, 1949); and the tetraploid cultivated raspberry variety of Hailshamberry ($2n = 28$) is now only available

as a triploid ($2n = 21$). We need not look only, as previously supposed, to replacement of the stock by self-sown seedlings, but can consider also the probability that somatic changes in chromosome numbers occur in the vegetative system, and with greater frequency than hitherto supposed.

SUMMARY

1. Serial cytological observations were made on one abnormal seedling from the thornless segregants of the F_2 progeny from crossing two tetraploid ($2n = 28$) Rubus species. The number of root-tip chromosomes was found to vary between 9 and 46 per cell, with the mode always at 35. The wide variation in chromosome number decreased with time, until there was almost stability at $2n = 35$. A vegetative (stipule) cell was $2n = 39$. The numbers lower than 35 were not due to terminal fusion.

2. Evidence from the number of satellites per cell suggests that not always were the same chromosomes being eliminated, although there is a general increase in satellite number with increased numbers of chromosomes per cell.

3. There was a correlation between the chromosome numbers and corresponding cell sizes within the aneuploid series. This may be associated with the incremental change of DNA following the addition or subtraction of individual chromosomes, which are highly similar in Rubus.

4. The cause of the instability was not definitely established. Its initiation is probably attributable to the egg mother cell, as Rubus pollen reacts sensitively to chromosome unbalance. No abnormalities were observed in the mitoses, and virus infection was not responsible for the instability.

5. Chromosome number instability of this plant is discussed in relation to other examples from the same and different genera: it is suggested that the changes in chromosome number known in various Rubi may have arisen somatically.

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Analysis of quantitative inheritance of body size in mice

IV. AN ATTEMPT TO ISOLATE POLYGENES*

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Large numbers and the weak action of individual units of polygenes usually involved in the inheritance of a quantitative genetic trait constitute basic difficulties in studying their effects. For these reasons the physical and biological properties of polygenes are poorly understood. In studying the effects of polygenes on body size we crossbred the Large and Small strains of mice which differ greatly in body size. Segregation of a rather large number of genetic factors was indicated in the F_2 generation (Chai, 1956). For a better understanding of the effects of polygenes, an attempt was then made to isolate a small number of them, introducing inheritance units from the Large to the Small mice and vice versa by repeated backcrossing and selection in two directions. The methods used and results obtained are the subject of this report.

MATERIALS AND METHODS

The data analysed in this paper are the body weights of 60-day-old mice from the Large and Small strains and their backcross generations. The development of these two strains has been reported elsewhere (Chai, 1956). At the time of the last backcross generations, mice of the parental strains, Small and Large, were at the twenty-fifth and fifteenth generations, respectively, of brother–sister mating. Selection for body size during the early development of these strains may have resulted in some increase in homozygosity in the loci determining body size. It is not likely that there may still be some residual heterozygosity in these loci in the Large mice as the H^2 locus does show some heterozygosity. Agglutination tests of blood cells with respect to the H^2 locus were made.‡ All the Small mice chosen for the test gave the same reaction while a very small portion of the Large mice reacted differently from the rest of them.

Backcrossing to the Large strain was carried to the fifth generation and to the Small strain to the seventh generation. During the course of backcrossing starting with the second generations selection was concurrently carried on by mating large-body-sized mice in one line and small-sized mice in another line in each backcross

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‡ The author is greatly indebted to Dr. Jack Stimpfling for typing the red blood cells.

to both Large and Small strains. Selection was started with the second backcross generation to the Large strain as well as to the Small strain. Therefore in the backcrosses to each parental strain, two 'paired' lines were formed. The breeding

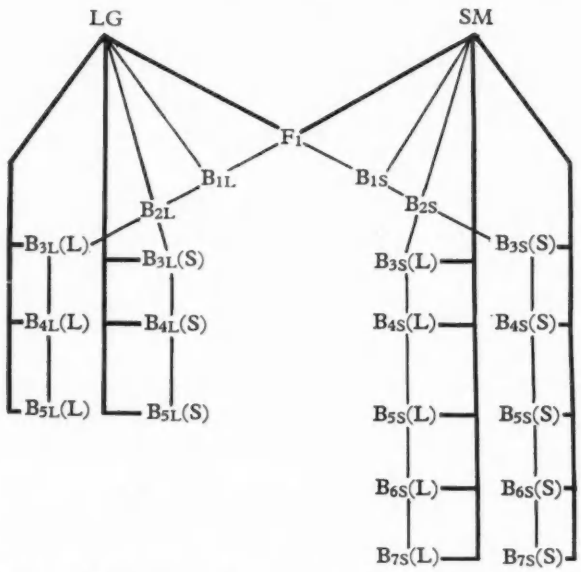

Fig. 1. Diagram of backcrossing and selection (see text for notations).

scheme is diagrammed in Fig. 1. The notations used in the diagram are given below:

LG = Large strain
SM = Small strain
B_{1L}, B_{2L}, ..., B_{5L} = 1st, 2nd, ... 5th backcross generations to the Large strain
B_{1S}, B_{2S}, ..., B_{7S} = 1st, 2nd, ... 7th backcross generations to the Small strain
(L) = mice in a generation selected for large body size
(S) = mice in a generation selected for small body size

For example, B_{3L}(L) refers to the group of mice in the third backcross generation to Large, selected for large body size. This breeding scheme is designed to introduce single inheritance units determining body size from one strain to the other. Each unit may contain one polygene or several which are closely linked. By repeated backcrossing to the Small strain and selecting for large body size, single factors with plus effects might be introduced from the Large mice to the Small mice. Thus it might be possible to observe the effect of single inheritance units from the Large on the Small background. Backcrossing to the Small strain, combined with selection for small body size, tests whether there are factors with minus effects which are present in the Large but not in the Small strain.

(It is realized that in this breeding scheme genes with large additive effects and free segregation as well as highly inbred strains are desirable. Because dominance, genic interaction, linkage or mutations can cause complexity and hinder the progress of isolation, completely satisfactory stocks for this type of investigation may be hard to obtain. Nonetheless, observations on the effects of rather small numbers of, if not single, genetic units acting on relatively homogeneous genetic backgrounds, as in the present study, are considered worth while.)

The management of the mouse colonies remained the same throughout the period of the experiment, and was the same as in the earlier studies (Chai, 1957). Litter sizes larger than eight were reduced to eight. All mice were weaned at 4 weeks of age and were weighed at 60 days. Starting with the third generation, all of the female parents were from the parental strains. This practice made unnecessary the correction for maternal influence, a difficult process which can hardly be carried out adequately.

For convenience of comparison between all backcross generations and the parental strains raised in different years, the means and variances for the mice of the parental strains and the first and second backcross generations previously published (Chai, 1956, 1957) are re-listed.

RESULTS

The means and variances on actual and logarithmic scales for the Large mice and their backcrosses are given in Table 1 and those for the Small mice and their backcrosses in Table 2. With successive backcross generations, the mean body weights of the mice regress toward the mean of their respective parental strain with greater rates of increment and declination in the early backcross generations to Large and to Small mice, respectively (Fig. 2).

Table 1. *Means and variances on actual and logarithmic scales for the Large mice and their backcrosses*

Genetic groups	No. of mice	$\bar{x}$	$\overline{\log x}$	s_b^2	s_w^2	$1000 s_{\log x, w}$
LG (1954–5)	65	37·35	1·5678	8·36	3·92	0·49
(1956)	116	37·44	1·5707	10·71	6·25	0·86
(1957)	259	36·03	1·5524	16·77	8·96	1·30
(1958)	160	38·50	1·5855	10·68	6·07	0·77
F_1	161	25·82	1·4075	5·47	1·28	0·36
B_{1L}	164	31·59	1·5007	4·15	6·70	1·25
B_{2L}	203	35·30	1·5443	9·02	8·97	1·36
$B_{3L}(L)$	210	35·70	1·5499	9·08	7·33	1·08
$B_{3L}(S)$	98	35·80	1·5514	5·34	9·95	1·48
$B_{4L}(L)$	131	38·38	1·5822	7·78	5·90	0·75
$B_{4L}(S)$	149	36·83	1·5625	13·12	9·91	1·37
$B_{5L}(L)$	212	39·26	1·5926	3·46	6·40	0·79
$B_{5L}(S)$	122	40·32	1·5939	5·34	6·40	0·74

The expected means without selection were calculated on the basis of additive

effects of the polygenes, and were plotted in Fig. 2, beginning from the third backcross generation. (The calculations were made by adding the mean of the previous backcross generation to the mean for the parental mice produced in the same year and dividing by 2.) The means for the B_S(S) did not show much difference from the means without selection, while the B_S(L) did. The means of the B_L generations fluctuated.

Attention should be focussed also on the differences of the means from those of the parental strains and between the (L) and (S) groups in each generation of each backcross (Fig. 2). In the backcrosses to the Large, there is practically no difference from the parental strain in the groups either selected for large (L) or

Table 2. *Means and variances of 60-day body weights on actual and logarithmic scales for the Small mice and their backcrosses*

Genetic groups	No. of mice	x	$\overline{\log x}$	s_b^2	s_w^2	$1000s^2_{\log x, w}$
SM (1953–4)	39	14·0	1·1434	0·74	0·65	0·62
(1955–6)	144	14·6	1·1623	0·52	1·00	0·89
(1957)	260	15·1	1·1775	1·33	1·14	0·94
(1958)	110	15·9	1·1961	3·12	1·53	1·15
F_1	161	25·0	1·3943	5·47	1·28	0·36
B_{1S}	154	19·4	1·2858	4·25	4·21	1·84
B_{2S}	158	18·6	1·2715	1·02	2·97	1·57
B_{3S}(L)	110	17·0	1·2258	2·70	2·70	1·33
B_{3S}(S)	57	15·8	1·1971	2·35	0·80	0·60
B_{4S}(L)	185	15·7	1·1923	0·81	2·06	1·58
B_{4S}(S)	55	15·4	1·1848	0·98	1·05	0·83
B_{5S}(L)	253	16·4	1·2119	0·64	1·64	1·15
B_{5S}(S)	122	15·4	1·1858	0·65	0·93	0·74
B_{6S}(L)	241	16·3	1·2114	1·13	1·24	0·87
B_{6S}(S)	228	15·1	1·1778	1·17	1·24	1·02
B_{7S}(L)	145	16·8	1·2218	1·30	1·54	1·03
B_{7S}(S)	64	16·1	1·2056	0·89	0·89	0·65

selected for small (S) body size after the second backcross generation (B_{2L}). Comparing the means between the (L) group and the (S) group in each backcross generation the differences were not in the expected order with respect to selection. However, in the Small backcrosses the means of the B_{2S}'s are much greater than that of their parental strain, SM. Although the mean of B_{3S}(S) appeared no different from that of SM, the mean of B_{3S}(L) is considerably greater than that of SM. In succeeding backcross generations, the means of the (L) groups continue to be larger (except B_4) than those of the corresponding (S) groups in each generation through the B_7, and also to be larger than that of the parental strain.

The means of the Small mice indicate a gradual, although slight, increase in body weights with lapse of time and advance of inbreeding. This tendency is not shown in the Large strain. The means of both the Large and Small mice for the year 1958 were greater than the respective means for the previous years. This may be considered due to an environmental effect.

Variances between litters and within litters in the parental strains as well as in the backcross generations are given in Tables 1 and 2 and illustrated in Figs. 3*a* and 3*b* on logarithmic scales. The within-litter variance (s^2) is considered to be the better estimate for a comparison of the genetic differences between the backcross generations and the parental strains, and the litter mean variance (s_b^2) is considered to be the better indication of environment effects on animals of the different genotypes, or of genotype-environment interaction between the genetic

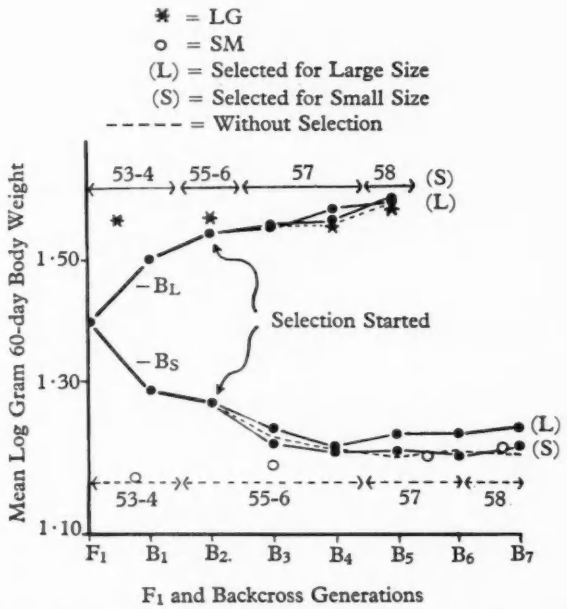

Fig. 2. Means of 60-day body weights on log scale for mice in the parental strains, F_1 hybrid and the backcross generations. The correspondence in the year of birth between the Small mice and their backcrosses is given by dotted lines and that for the Large mice and their backcrosses by solid lines.

groups (see Chai, 1956). Therefore, for comparison between the genetic groups, s_w^2 is used for evaluation of genetic differences and s_b^2 for variation in response to environment (genotype, environment interaction). (It is realized that there is a genetic component in the s_b^2 which varies with the number of heterozygous loci and differences between loci in each group, or both, but for the data presented here, variation from this source is relatively small in comparison with that from the environment.)

There may be a slight tendency for the within-litter variance to decline with the advance of backcrossing in (L) groups in the B_L generations, but this tendency may be somewhat overshadowed by the great fluctuation of the variances between years, as shown in the LG. In the B_{3L} and B_{4L}, the variances appear to be greater

in the (S) groups than in the (L) groups, but in the B_{5L} generation the variances in the two groups are similar in magnitude. In the B_S generations, the differences in variances between generations and between the (L) and (S) groups are unlike those in the B_L. The variance declines with successive backcrossing in the (L)

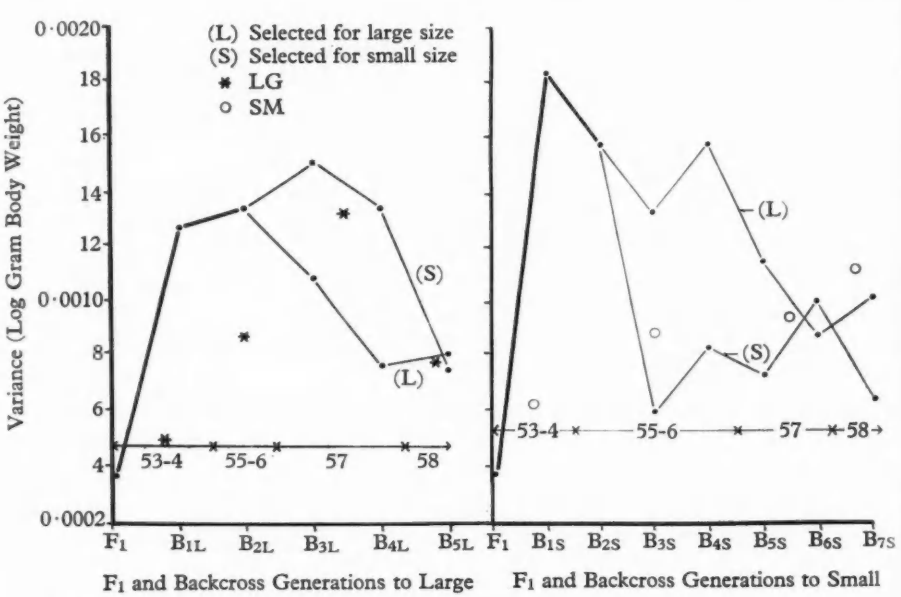

Fig. 3*a*. Variances of 60-day body weights in logarithm for mice of the Large strain and their hybrid generations. The correspondence in the year of birth between the Large mice and those in their hybrid generations is given by the solid lines with arrows.

Fig. 3*b*. Variances of 60-day body weights in logarithm for mice of the Small strain and their backcross generations. The correspondence in the year of birth between the Small mice and mice in their hybrid generations is given by the solid lines with arrows.

groups but not in the (S) groups. Furthermore, in comparison with SM, the variances in the (L) groups are fairly large, while those in the (S) groups are small and distributed within the lower range of the variance for the SM.

DISCUSSION

The results with respect to the distribution of means and variances in the backcross generations seem to support the earlier interpretation that genes responsible for large body size are dominant over those for small body size (Chai, 1957). In the means, a differential remained between SM and the advanced generations of B_S(L), but not between LG and B_L(S), and a differential persisted between the (L) and (S) groups in the B_S but not in the B_L groups. It is evident that selection had no effect in the backcrosses to LG. This can fit with the dominance hypothesis

in that the heterozygotes are hardly distinguishable phenotypically from homozygous dominants but are distinguishable from homozygous recessives.

The decrease in variation following the advance of backcrossing in the $B_S(L)$ indicates a gradual reduction in the number of the genes for large body size introduced from the Large strain. In the $B_S(S)$ the variance did not decrease beyond the third generation. This may be easily explained by the fact that from the third generation the $B_S(S)$ mice were genetically very similar, as far as body size was concerned, to the parental Small mice, indicated by the similarity of means. The variances of the Large backcrosses were entirely different. There was no definite trend, other than random variation, which is probably a reflection of environmental effects and possibly of sampling errors. Although the number of minus genes from the Small strain was expected to decline proportionately in each backcross generation, the variances did not indicate this tendency. This could be explained by the effect of dominance of the plus genes over the minus genes and selection, even if no other factors were involved.

The interpretation of dominance of genes with plus over those with minus effects is in agreement with the results obtained by others (MacArthur, 1944; Falconer & King, 1953). It is, however, not fully justified in view of the fact that the mean of the F_1 hybrids was not much greater than the mid-parent value. The overall situation seems to suggest that the relative effects of dominance and additivity of the polygenes may not be constant within the range of genotypes. In other words, the interactions between genes are not additive with respect to the number of participating genes. It may also be that the Large genes have a different magnitude of dominance.

Clear-cut conclusions certainly cannot be made with respect to isolation of genetic determinants on the genic level on the basis of the data presented. It may be tentatively stated, however, that single, or rather small numbers of, inheritance units have been introduced from the large mice to the small mice as shown by the larger size of $B_{7S}L$ over SM. More definite conclusions possibly will be reached by establishing inbred lines from the last backcross generations, crossing between these lines and transplanting tissues between and within them; such studies are in progress.

SUMMARY

The isolation of single inheritance units affecting body size in mice has been attempted. By using Large and Small mice as the parental strains, a breeding scheme has been carried out by repeated backcrossing to each strain with selection for both large and small body sizes in each backcross. The selections were started from the second backcross generations. The Small backcross was carried to the seventh generation and the Large to the fifth. Based on analysis of the means and variances for the parental strains and the backcross generations, it is tentatively concluded that a small number of, if not single, inheritance units may have been introduced from the Large to the Small mice. The 'large' genes appear to be dominant over the 'small' genes.

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Genetical studies on the skeleton of the mouse

XXVII. THE DEVELOPMENT OF OLIGOSYNDACTYLISM

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INTRODUCTION

It has been shown repeatedly in this series of papers that in many 'skeletal' mutants the skeleton is only secondarily involved. Indeed, contrary to what one might expect from the form-giving solidity of the adult skeleton, the membranous skeleton is vulnerable and easily disturbed by anomalies in its vicinity. This is strikingly illustrated by the case of syndactylism (*sm*/*sm*) in the mouse (Grüneberg, 1956, 1960) where fusions between adjacent digits (and occasional tail kinks) can be traced back to a hyperplasia of the epidermis which is present before there is any sign of blastemata in the limb buds. The case of Oligosyndactylism (*Os*/+) to be described in this paper is similar in that the limb skeleton seems to be only secondarily involved. However, the underlying mechanism is quite different as is the anatomical situation in the adult animal.

ADULT ANATOMY

As described in detail previously (Grüneberg, 1956), all four feet of *Os*/+ heterozygotes are regularly affected, the hind limbs more severely than the fore limbs. Syndactylism is usually confined to digits II and III. Hard-tissue fusions between these digits generally start at the basal phalanges and thence spread distally; in more severely affected feet, the metacarpalia or metatarsalia II and III are also more or less completely fused. Loss of a digit may thus arise by fusion between digits II and III. However, digit II is often thinner than normal and may vanish without fusion with digit III. Sometimes blastemal material of digit II seems to become incorporated in digit I which may then be duplicated; the resulting loss of digit II associated with duplication of digit I leads to a pentadactyl though very abnormal limb. Metacarpale V and metatarsale V are nearly always proximally fused with metacarpale IV and metatarsale IV respectively in a state of abduction; this is partly attributable to a reduction of the normal locking mechanism between these elements, and partly to lack of proximal support by the neighbouring carpalia and tarsalia respectively which are reduced in size. Extensive fusions regularly occur in carpus and tarsus. All fusions between metacarpalia and metatarsalia are secondary, i.e. they result from the concrescence of elements at first laid down as separate cartilages. However, cuboideum and cuneiforme III are primarily fused, i.e. they are represented by a single cartilaginous element from the beginning, and the same

applies to some, at least, of the fused phalanges. No other skeletal abnormalities have been discovered.

EXTERNAL FEATURES OF *Os*/+ EMBRYOS

A description from the 14-day stage onwards has been given previously. The hands and feet of 13-day *Os*/+ embryos (Fig. 1) can easily be distinguished from those of their normal litter-mates by a flattening of the preaxial border (on top in

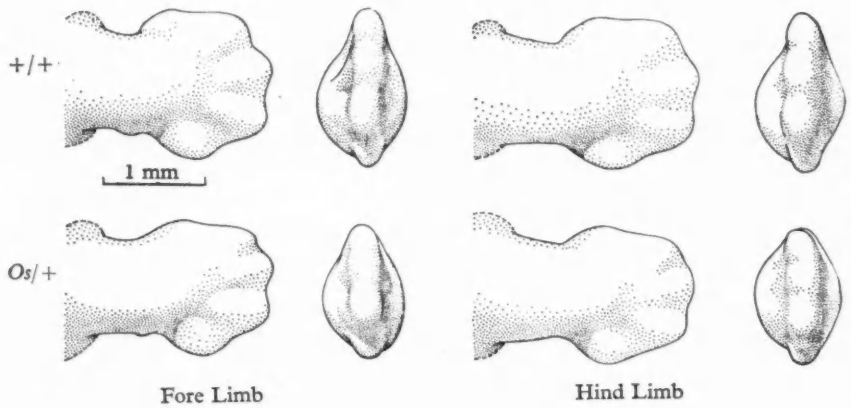

Fig. 1. Right fore and hind limbs of 13-day +/+ and *Os*/+ embryos (litter-mates). Camera lucida drawings.

Figs. 1–4) whereas the rest of the foot is little, if at all, affected. In cross-section, *Os*/+ limbs are about as thick as those of normal embryos: the defect on the preaxial border is thus not compensated by excess material elsewhere and represents a real reduction in size of hands and feet.

The same situation is already encountered in the 12-day stage (Fig. 2). The

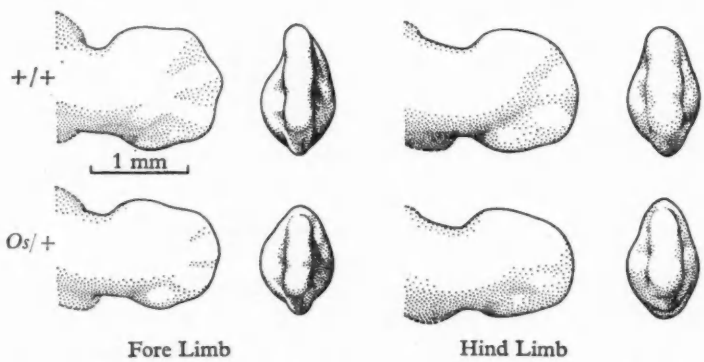

Fig. 2. Right fore and hind limbs of 12-day +/+ and *Os*/+ embryos (litter-mates). Camera lucida drawings.

preaxial border of hands and feet is flattened to just about the same extent as in the 13-day stage.

In the 11-day stage (Fig. 3), the foot plate of the fore limbs of normal mouse embryos is circular in outline and can just be distinguished from the rest of the

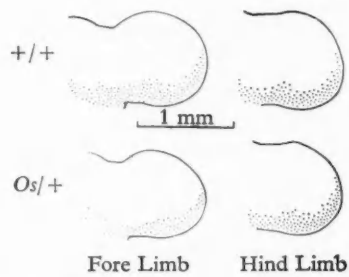

Fig. 3. Right fore and hind limbs of 11-day embryos (litter-mates). Top row putative normal (+/+); bottom row putative *Os*/+ embryo. Camera lucida drawings.

limb; in the hind limbs there is not yet any separation into foot and leg. In segregating litters, *Os*/+ embryos can be distinguished from the rest by a flattening of the preaxial border of the fore limbs so that the outline is ovoid rather than circular; in the hind-limb buds a corresponding difference seems to be just appearing, but, if real, it is still very slight. The difference between putative normal and putative *Os*/+ embryos is more conspicuous when the outlines of the limbs are superimposed on each other (Fig. 4). The incidence of the two types of embryos is in reasonable

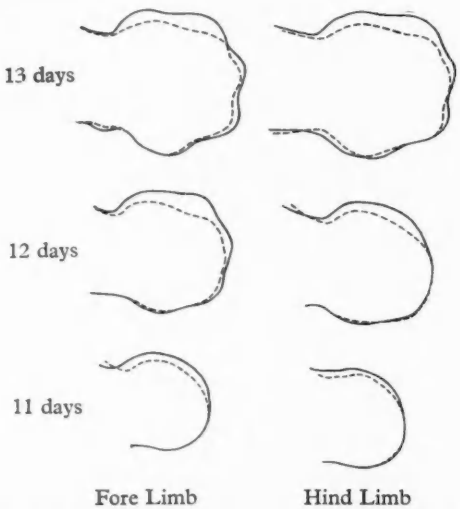

Fig. 4. Outline drawings of the right fore and hind feet of normal (solid lines) and *Os*/+ embryos (broken lines) superimposed on each other. Same specimens as in Figs. 1–3. Note that, throughout, the preaxial area of the *Os*/+ foot plates (on top in drawings) is reduced whereas the remainder of the feet is virtually normal.

agreement with the Mendelian expectation. Four *Os*/+ × *Os*/+ litters included fourteen putative *Os*/+ and four putative +/+ embryos; one *Os*/+ × +/+ litter included similarly three *Os*/+ and four +/+ embryos. If the identification of the *Os*/+ embryos at the 11-day stage is correct, the flattening of the preaxial border is detectable as soon as the foot plates become distinguishable as separate structures, i.e. before any membranous skeleton can be detected in them by standard histological techniques.

An attempt to identify younger (9½–10-day) *Os*/+ embryos by external inspection has not been successful. Eleven embryos from *Os*/+ × +/+ and twenty-seven from *Os*/+ × *Os*/+ matings showed no obvious differences attributable to segregation in the shape of the limb buds.

SECTIONED *Os*/+ EMBRYOS

The sectioned material is summarized in Table 1. In each case but one, all four limbs were sectioned, so that the total number of limbs examined is 183. Normal and *Os*/+ embryos were litter-mates throughout.

Table 1. *Normal and* Os/+ *embryos sectioned*

Age	Litters	+/+	*Os*/+	Total
15	1	1	2	3
14	3	3	4	7
13	3	5	8	13
12	5	9	10	19
11	1	2	2	4
Total	13	20	26	46

The situation encountered in the limbs of 13- and 12-day-old embryos (Figs. 5–10) is similar in its main features though it somewhat differs in degree. Nearly always, digits II and III are closer together than in normal feet and tend to be parallel to each other, whereas normally these elements include an angle of roughly 20°. The fore limbs (left half of Fig. 5, Fig. 7) tend to deviate less from the normal pattern than the hind limbs (right half of Fig. 5, Fig. 6), as in the adult animal. The angle included by digits III and IV also tends to be rather smaller than normal, but the difference is much less; this again is in agreement with the fact that syndactylism between these digits happens only rarely. Digits IV and V are never webbed and the angle included is about normal. It is obvious that the crowding together of digits II and III (and, to a much lesser extent, of digits III and IV) is the direct consequence of the fact that these digits develop in a foot plate which is reduced in size preaxially. In the 13-day embryo, chondrification has started in metacarpalia II–IV, but not yet in the corresponding metatarsalia; there is no chondrification in the feet of 12-day embryos. As the near-parallel arrangement of digits II and III is clearly present as soon as the blastemata become visible (see particularly Fig. 9), it is obvious that the cartilaginous and indeed the osseous skeleton merely repeats a pattern already present in the membranous skeleton;

and that the membranous skeleton itself is forced to deviate from normality by the pre-existing flattening of the preaxial border of the foot plate: for the shape anomaly

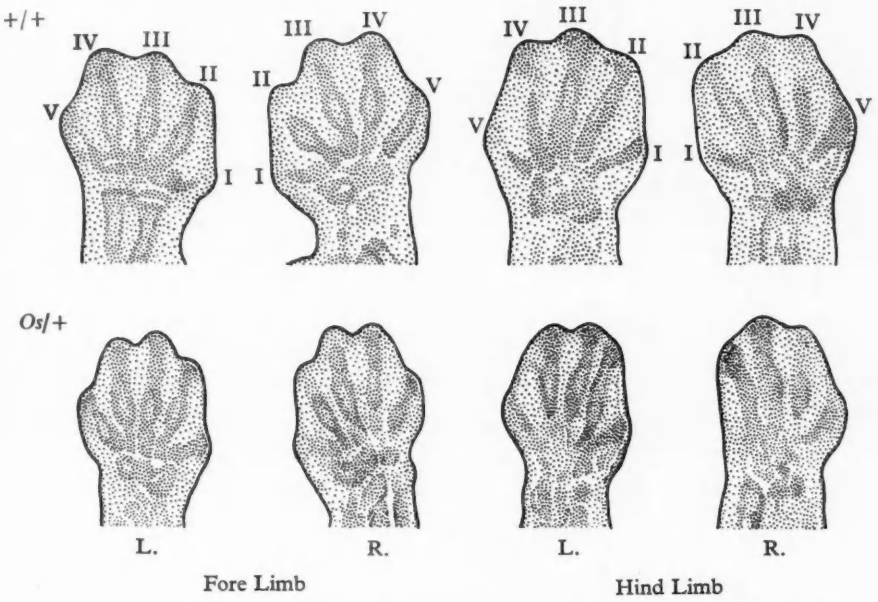

Fig. 5. Sections through the feet of a normal and an *Os*/+ embryo (13-day-old litter-mates, C.R.L. 8·6 and 9·0 mm. respectively). Figs. 5–10 are drawings based on microphotographs. Final magnification × 21.

of the foot plates can be detected in the 11-day stage, i.e. before any condensation of mesenchyme has taken place.

Evidently, a moderate degree of approximation between digits II and III will lead to soft-tissue syndactylism. When the two elements are closer together (such as in the hind limbs, Fig. 5), a common basal phalanx would presumably have been

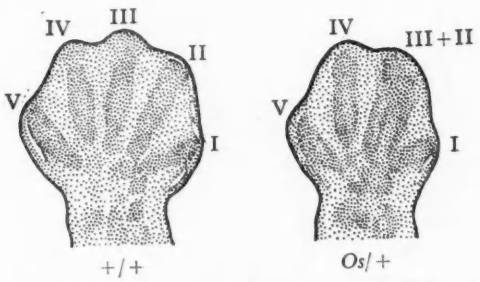

Fig. 6. Sections through the left hind limbs of a normal and an *Os*/+ embryo (13-day-old litter-mates).

formed. It is also understandable that only in such cases in which all the phalanges are fused will the metacarpals or metatarsals be close enough together to coalesce

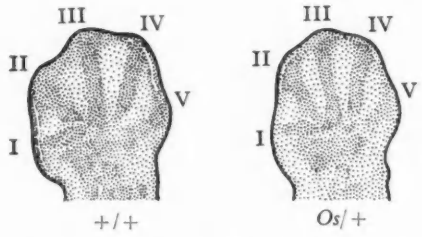

Fig. 7. Sections through the right fore limbs of a normal and an *Os*/+ embryo (12-day-old litter-mates, C.R.L. 7·2 and 7·9 mm. respectively).

with each other. Such a state is apparently reached in the limb shown in Fig. 6 which would almost certainly have become a case of oligodactylism by fusion between digits II and III.

The flattening of the preaxial border of the limbs leads not only to a crowding of

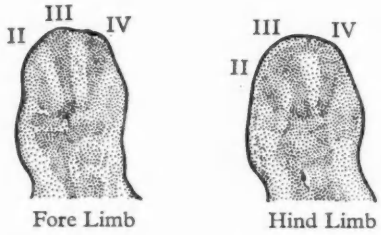

Fig. 8. Sections through the right fore and hind limbs of an *Os*/+ embryo, 12 days old (C.R.L. 7·7 mm.).

the blastemata, but also to a reduction of the mesenchymal material available for skeletal development. It seems that in many cases mesenchyme normally used for the interdigital area between digits II and III is incorporated into digit II which is then of normal or near-normal calibre. In other instances, digit II is clearly thinner

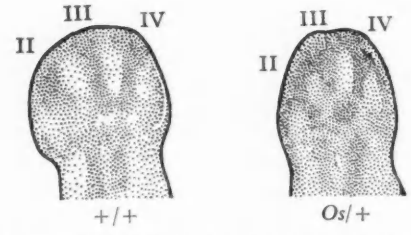

Fig. 9. Sections through the right hind limbs of a normal and an *Os*/+ embryo (12-day-old litter-mates, C.R.L. 7·4 and 7·2 mm. respectively).

than digit III, as in Fig. 6 and in Fig. 10 (see also Figs. 12 and 13, p. 128, in Grüneberg, 1956). Such small elements have a tendency to fuse with their neighbours (as in Fig. 6 in which this process is almost complete). Where small blastemata remain independent, they may fail to chondrify because they fall below a critical threshold of size. Perhaps, in some cases, small blastemata may secondarily disintegrate again by migration of mesenchyme cells towards vigorously-growing larger neighbours (competition between blastemata in the sense of Tschumi, 1954). In either of these two cases, true oligodactylism of digit II is clearly due to the reduction of mesenchymal material in the preaxial area of the foot plates.

In the large majority of cases, digit II is formed closer than normal to digit III. An exception is shown in the right hind limb of Fig. 10 where digit II is abnormally close to digit I. Evidently this is a situation which may lead to oligodactylism of digit II associated with polydactylism of digit I as described previously (Grüneberg,

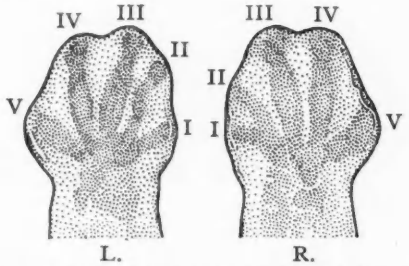

Fig. 10. Sections through the hind limbs of a 13-day-old *Os*/+ embryo (litter-mate to those in Fig. 5; C.R.L. 8·3 mm.).

1956); the material for digit II coming under the 'influence' of digit I and forming an additional hallux rather than a digit II.

Attempts to discover the cause of the preaxial reduction of the foot plates have not been successful. It is probably safe to say that there are no gross pathological lesions which could be held responsible. There is no pathological cell pyknosis, and the vascular system and the apical ectodermal ridge do not appear to be grossly abnormal. On the other hand, the possibility of course remains that a more detailed study, particularly with the aid of more refined (histochemical, etc.) techniques, may be more successful.

DISCUSSION

The first anomaly of *Os*/+ embryos discovered is a reduction of the preaxial margin of the foot plates in the 11-day stage, i.e. before any condensation of mesenchyme has taken place. The whole complex morphology of the adult limb skeleton can be understood as a consequence of this simple initial disturbance.

The preaxial reduction of the foot plates diminishes the space and the material available for the formation of the foot skeleton. Most affected is digit II which is usually shifted towards digit III; depending on the degree of this shift, this leads to webbing of the soft tissues, to cartilaginous and subsequently osseous fusions

between phalanges, and ultimately, when metacarpals and metatarsals are also involved, to oligodactylism by fusion between digits II and III. Sometimes, digit II is deflected towards digit I, and then differentiates into a hallux. Where digit II is reduced in calibre or altogether absent as a hard structure, this is clearly due to a reduction of the mesenchyme available. Presumably the same causes are also responsible for the extensive fusions in carpus and tarsus. Unfortunately, the obscurity which shrouds the early development of the mammalian carpus and tarsus (Holmgren, 1933, 1952; Schmidt-Ehrenberg, 1942; Milaire, 1956) makes direct proof virtually unobtainable.

A condition strikingly similar to *Os*/+ has been obtained by Tschumi (1954), who treated the early limb buds of the clawed toad (*Xenopus laevis*) by means of a mitotic poison; the foot plates which subsequently developed were reduced preaxially, but not postaxially, and showed all stages from webbing of toes to complete fusion, particularly of digits I and II; in addition, toes may be reduced in calibre down to vanishing point; tarsal fusions were also common. Evidently, organisms as different as *Xenopus* and the mouse react to diminution of the preaxial region of the feet in much the same way; i.e. they use space and material at their disposal according to the same rules which obtain in normal development. Tschumi suggests that, in *Xenopus*, a generalized reduction of the limb is secondarily localized on the preaxial side by a process of competition: the digits which differentiate first (III–V) seem to use up about as much mesenchyme as if they developed in a normal limb and thus do not leave enough material for digits I and II.

The same idea has more recently been discussed by Forsthoefel (1959) in relation to the development of the gene for luxoid (*lu*/*lu*) in the mouse. 'The reduction in limb bud size would of itself be expected to result in preferential loss of the preaxial portions of the limb since condensation of blastemata begins in the postaxial portion. Thus the fibula and the lateral digits are first laid down. If no further material is available, the preaxial tibia and the medial digits will be missing. This may explain also why in the mouse anomalies in limb development are almost always preaxial. Excess or defect of material would be expected to affect the elements last laid down.' While Tschumi (1954) may well be right for *Xenopus*, I rather doubt whether Forsthoefel is for the mouse: in the first instance, in the mouse digits II–IV seem to be laid down first (see, e.g., Fig. 9 above), and both marginal rays follow later; hence Forsthoefel's argument could apply to both preaxial and postaxial defects. Moreover, in *Os*/+ at any rate, and probably in most of the entities to be discussed presently, defect or excess material can be detected on the preaxial margin before the onset of blastema formation; hence the anomaly is localized in the foot plate from the start, and not only secondarily as the result of competition for a limited amount of mesenchyme. Postaxial defects, incidentally, are not very rare in the mouse. There is oligodactylism (*ol*/*ol*; Freye, 1954), postaxial polydactylism (*tu*/*tu*; Center, 1955) and postaxial hemimelia (*px*/*px*; T. C. Carter, unpublished).

Inherited preaxial limb anomalies have been described in a variety of animals. Those studied embryologically include the genes for luxate (*lx*/*lx*; Carter, 1954) and for luxoid (*lu*/*lu*; Forsthoefel, 1959) in the mouse in which defects of the zeugo-

podium are associated either with defects or with excess formations (polyphalangy, polydactylism) of preaxial digits. Preaxial polydactylism as part of a complex syndrome has been studied in the guinea-pig (Scott, 1937) and, as a separate entity, in the mouse (Chang, 1939), in the cat (Danforth, 1947) and in the chicken (Zwilling, 1956; Zwilling & Hansborough, 1956; and earlier authors). In all these cases there is defect or excess of limb material before condensations of mesenchyme appear in it. Evidently, the material is used up in much the same way as in a normal limb, no matter whether there is too little or too much of it. As Danforth (1947) put it in the case of polydactylism in the cat, '. . . the only difference between potentially polydactyl and normal specimens is the amount of undifferentiated tissue on the preaxial border of the limb. It seems not impossible that induction of an early excess in the number of cells at this point may be the chief, perhaps only, direct effect of the mutant gene, and that all other associated morphological deviations are merely secondary to this initial volumetric increase.' *Mutatis mutandis*, the same explanation would seem to apply to *Os*/+ in the mouse.

For the present, the cause of the preaxial reduction of the foot plates of *Os*/+ embryos remains obscure. Zwilling & Ames (1958) have suggested that in polydactylism, the excess growth may be due to an anomalous distribution of the hypothetical 'maintenance factor' for the apical ectodermal ridge. Purely formally, a similar concept could account for the present case. But there is so far no supporting evidence for such an hypothesis.

Many authors have noticed that under pathological conditions, cartilaginous elements have a tendency to fuse with each other. The reason for this seems to be obscure. Where a cartilage of reduced size fuses with an adjacent element, the explanation may be found in Tschumi's (1954) suggestion that a vigorously growing blastema may compete with a smaller neighbour for mesenchyme cells; if such cells migrating across from one to the other are overtaken by the process of chondrification, fusion between the two elements will result. Sometimes cartilages of about normal dimensions fuse with each other when they are located abnormally close to each other. In such cases, the blastemata, instead of attracting mesenchyme cells from the intervening area, seem mutually to attract cells from each other, with a similar result. Fusions between elements which have arisen in a continuous blastema (such as carpals and tarsals) are presumably due to a different mechanism.

SUMMARY

The complex anatomy of the adult limb skeleton of *Os*/+ mice is attributable to a reduction of the preaxial margin of the foot plates. This is detectable, in the fore limbs, in the 11-day stage, i.e. before condensations of mesenchyme have taken place. The involvement of the skeleton is thus secondary to an earlier defect in the foot plates. The cause of the latter has not been discovered.

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Heterogeneity in recombination frequencies in *Neurospora crassa*

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1. INTRODUCTION

In the past diverse wild-type stocks of *Neurospora crassa* have been used by workers. Lindegren (1932*a*) used only two wild strains of opposite mating-type, but Beadle & Tatum (1945) also introduced the Abbott and Chilton stocks in order to maintain vigour on intercrossing (Beadle—personal communication). Emerson & Cushing (1946) and St Lawrence (De Serres, 1958*b*) produced new 'wild-types' by hybridizing Abbott and Lindegren strains. The origin of the wild-type strains and the wild-type ancestry of the originally isolated biochemical mutant strains is known (Table 1). However, as few records have been kept of re-isolations, the laboratory stocks of today are of unknown but highly mixed wild-type ancestries.

In their linkage studies Houlahan, Beadle & Calhoun (1949) pooled data from several crosses involving a single locus. When these data were tested, marked heterogeneity was present in the centromere distance of the majority of the loci (Frost, 1955*b*). The first report of substantial strain differences in the centromere distance of a locus was by Teas (1947). Barratt, Newmeyer, Perkins & Garnjobst (1954), Holloway (1954) and Stadler (1956*a*) reported heterogeneity in centromere distances based on ordered tetrads. In random spore analyses, discrepancies or heterogeneity in recombination frequencies between crosses of the same linked markers were reported by Mitchell & Mitchell (1954), Frost (1955*b*), Rifaat (1956, 1958), Mitchell (1958), De Serres (1958*a*) and Perkins (1959).

Barratt (1954) summarized the data on heterogeneity in centromere distance of loci including instances from almost every linkage group and pointed out that this was associated with the presence in the crosses of an Abbott stock as opposed to a Lindegren stock. Significant reduction of centromere distance over that from crosses of two Lindegren stocks occurred in crosses of Abbott 4 and Lindegren derivation for *mt*, *al-1*(4637T), *me-3*(36104), *nt*(39401) and *chol-1*(37903) but not for *inos*(37401), and in crosses of Abbott 12 and Lindegren derivation for *me-5*(9666) but not for *mt*, *arg-6*(29997) and *me-5*(36105). Significant increases in centromere distance over the Lindegren value occurred in crosses of Abbott 12 and Lindegren derivation for *pab-1*(1633) and for *mt* when either Abbott 4 or 12 was crossed with a mutant of Abbott and Lindegren derivation. Frost (1955*b*) analysed in detail the data, including those kindly supplied by Dr R. W. Barratt, on heterogeneity in centromere distances and investigated further into the problem. Since this work

was not published and further experimental work has been carried out, the entire work is presented here. Heterogeneity in recombination frequencies has caused confusion in the study of the formal genetics of *Neurospora*. It is hoped that this report will be of value in clarifying the situation.

2. METHODS AND MATERIALS

Nomenclature

The system of designating strains will be that of Barratt *et al.* (1954), except that mating-type will be designated *mt* instead of *sex*, and strain 34508, *aur* instead of *al-1*.

Strains

The following strains were used, the origin being indicated by name after each strain.

Wild-types: Lindegren 1*A*, Lindegren 25*a*, Abbott 4*A*, Abbott 12*a*. (R. W. Barratt)

ad-3(35203), adenine. (R. W. Barratt)

al(G2), albino, believed allelic or pseudo-allelic with *aur*(34508). (B. M. Elliott)

al-2(15300), albino. (D. G. Catcheside)

arg-3(30300), arginine. (30300–68, D. G. Catcheside; R30300, A. M. Srb)

arg-6(29997), arginine. (D. G. Catcheside)

asco(37402), also known as *lys*. Requirement for lysine also ascospore abortion. (M. B. Mitchell)

nic-1(3416), nicotinic acid. (D. G. Catcheside)

Methods

The methods used were essentially those of Beadle & Tatum (1945). Crosses were made on the minimal reproductive medium devised by Westergaard & Mitchell (1947). Comparatively immature asci were dissected in an aqueous solution containing 1·5% w/v sodium hypochlorite and 1·5% v/v ethyl alcohol and were allowed to ripen for 10 days before heat treatment. To eliminate possibility of error, mating-type tests were carried out against standards of opposite mating-type. Inocula from each unknown culture was transferred into duplicate $3 \times \frac{3}{8}$ inch test-tubes; drops of conidial suspensions of standard *A* and *a* were then added to one and the other of the duplicates respectively.

3. SELECTION AND TREATMENT OF DATA

Only those data have been selected in which the wild-type ancestry of the strains crossed is known with certainty or was reported precisely. In addition the data must contain full details, for each individual cross, of the parentage of the cross together with the number of second-division segregations for the locus concerned, the total number of asci analysed and preferably also the total number of asci dissected since poor germination may give rise to biased results (Whitehouse, 1948).

Data fulfilling these essential requirements have been grouped according to wild-type ancestry of the crosses and each group tested statistically by means of the heterogeneity chi-squared test. If homogeneous, the totals of the first- and second-division segregations for a given locus of a certain ancestry type were compared by means of a two-by-two chi-squared test with the appropriate totals from crosses of pure Lindegren ancestry since the latter has been chosen as the standard wild-type ancestry against which to compare the behaviour of the other wild-type strains.

The origin or derivation of the wild-type strains and the wild-type ancestry of the mutant strains is given in Table 1. In some crosses of a given ancestry type,

Table 1. *Origin of the wild-type strains and wild ancestry of the mutant strains of* Neurospora crassa

Strain	Origin from	Ref.*
Lindegren *A*, Lindegren *a*	Material isolated by B. O. Dodge	1
1*A*, 10*a*, 19*a*, 25*a*	Lindegren *A* × Lindegren *a*	2
Abbott 4*A*, Abbott 12*a*	Material isolated by E. V. Abbott in Louisiana	2
Chilton *a*	Material isolated by St John P. Chilton in Louisiana	2
E5256*A*	Abbott 4*A* × 25*a*	3
E5297*a*	Abbott 12*a* × 1*A*	3
73*a*, 74*A*	E5256*A* × E5297*a* (P. St Lawrence)	4
308–952, 1274–26585	Lindegren *A* × Lindegren *a*	2
26586–35000	1*A* × 19*a*	2
35001–40100	1*A* × 25*a*	2
40101–44000, 60501–63000	Abbott 4*A* × Abbott 12*a*	2
44001–49000, 63001–*c.* 80000	Abbott 4*A* × 25*a*	2
49001–60500	Abbott 4*A* × Chilton *a*	2

* 1. Lindegren (1932*a*); 2. Beadle & Tatum (1945); 3. Emerson & Cushing (1946); 4. De Serres (1958*b*).

opposite mating-types of the same mutant strain were used. Beadle & Tatum (1945) stated that the mutant strains were each established from a single ascospore resulting from crossing irradiated material with a wild-type strain. Crosses were then made between these mutant strains and the wild-type from which they were derived. The author assumes therefore that it was from these latter crosses that both mating-types were obtained. In the case of mutant strains 308 to 40100 (Table 1) all such crosses would be of pure Lindegren ancestry. However, as regards mutant strains 40100 to 80000, it is not clear with which wild-type parent they were crossed in order to isolate both mating-types. The author has thus assumed that the ancestry of these strains is that given in Table 1. This may be an oversimplification of the wild-type ancestries involved but it does not fundamentally alter the situation. The data have been analysed where necessary to determine if opposite mating-types of the same mutant strain give significantly different results.

Besides data not fulfilling the essential requirements listed above, pooled data

from an unreported number of crosses and data from strains known to carry chromosome aberrations have been rejected.

4. HETEROGENEITY IN THE CENTROMERE DISTANCE OF THE MATING-TYPE LOCUS

The data given in Table 2 are arranged according to the wild-type ancestry of the strains crossed. The centromere distance of the Abbott 4 × Lindegren crosses shows a significant reduction from any of the other distances listed in the Table. The 'backcrosses' (Abbott 4 × Lindegren) × Lindegren show a significant decrease in centromere distance ($\chi^2 = 5{\cdot}210$, $n = 1$, $P = 0{\cdot}02$–$0{\cdot}05$) over the complementary backcrosses (Abbott 4 × Lindegren) × Abbott 4. The E5256 × Lindegren crosses are of (Abbott 4 × Lindegren) × Lindegren ancestry and show as expected no significant difference ($\chi^2 = 0{\cdot}403$, $n = 1$, $P = 0{\cdot}50$–$0{\cdot}70$) from the crosses of the latter ancestry type. The centromere distance of the (E5297 × Lindegren) selfed crosses is not significantly different ($\chi^2 = 0{\cdot}860$, $n = 1$, $P = 0{\cdot}30$–$0{\cdot}50$) from the E5297 × Lindegren value. However, Houlahan *et al.* (1949) listed a cross E5297*a* × 85902*A* which is probably of (Abbott 4 × Lindegren) × E5297 ancestry, giving a centromere distance for *mt* of 24·3 ± 4·11 (18/37) which is significantly increased ($\chi^2 = 5{\cdot}881$, $n = 1$, $P = 0{\cdot}01$–$0{\cdot}02$) over the E5297 × Lindegren value. It is the highest centromere distance of *mt* yet recorded in *N. crassa*.

It may be concluded from Table 2 that by grouping the crosses according to wild-type ancestry, heterogeneity in the centromere distance of *mt* is eliminated within any group.

Furthermore, the centromere distances of *mt* show similar values in crosses of Chilton compared with Lindegren ancestries but not in crosses of Abbott compared with Lindegren ancestries. Thus heterogeneity results from mixed wild-type ancestries involving an Abbott strain; significant increases over the Lindegren value being associated with an ancestry involving Abbott 12, while both significant increases and reductions result from the presence of Abbott 4. Therefore Abbott 4 is genetically different from Abbott 12, this being especially apparent in a direct cross to a Lindegren strain. As vegetative reproduction appears to predominate in nature, genetic differences between clones might well arise.

However, Lindegren *A* might differ genetically from Lindegren *a* rather than Abbott 4*A* from Abbott 12*a*. Against this supposition are the facts that the Lindegren strains used to obtain the above data have been inbred several times whereas the Abbott strains have not (Beadle & Tatum, 1945).

Furthermore, the author has intercrossed 1*A* with 25*a* and backcrossed some of the progeny to 1*A* for six successive backcrosses. Fertility is slightly reduced and some small variation in spore size results. When Abbott 12*a* was intercrossed with 1*A* and some of the progeny backcrossed to Abbott 12*a*, segregation occurred for ascospore abortion. Normal progeny were selected each time and backcrossed to Abbott 12*a* for five successive backcrosses. In each backcross generation, normal to aborted crosses occurred in approximately an equal ratio but there was much

Table 2. *The centromere distance of* mt *arranged according to wild-type ancestry of the crosses*

Data source*	Wild-type Ancestry†	No. of crosses	2nd-div. asci	Total asci	Het. χ^2	P(%)	Centromere distance	$2 \times 2\chi^2$ with (L × L)	P(%)
1, 2, 3, 4, 5	L × L	39	108	827	34·981	‡	6·53 ± 0·59	—	—
2, 4, 6, 7	A4 × L	13	13	282	10·085	50–70	2·31 ± 0·62	15·445	< 1
4	(A4 × L) × L	17	42	298	15·370	30–50	7·05 ± 1·01	0·203	50–70
4	(A4 × L) × A4	8	32	140	4·757	50–70	11·43 ± 1·77	9·283	< 1
4	E5256 × L	3	11	64	2·704	20–30	8·59 – 2·36	0·875	30–50
2, 4, 6, 8	A12 × L	9	22	193	9·598	20–30	5·70 ± 1·14	0·388	50–70
3, 4	E5297 × L	5	37	134	2·173	70–80	13·81 ± 1·93	19·062	< 1
3	(E5297 × L)⊕	10	55	169	11·133	20–30	16·27 ± 1·80	38·925	< 1
4	(A4 × L) × A12	4	20	77	0·623	80–90	12·99 ± 2·50	9·666	< 1
4	C × L	2	6	50	3·834	5–10	6·00 ± 2·30	0·047	80–90
4	(A4 × L) × C	3	7	41	2·361	30–50	8·54 ± 2·94	0·548	30–50
4	(A4 × C) × L	4	7	86	4·879	10–20	4·07 ± 1·47	1·713	10–20

* 1. Lindegren (1932*b*, 1936); 2. Buss (1944); 3. Holloway (1953); 4. Houlahan *et al.* (1949); 5. Regnery (1947); 6. Srb (1946); 7. Doermann (1946); 8. Frost (1955*b*).

† A = Abbott; C = Chilton; E = Emerson; L = Lindegren; ⊕ = selfed.

‡ Not significant.

variation in spore size from minute colourless to giant black spores. When Abbott 4*A* was similarly investigated (S. Lavigne, personal communication), sterility in the first and subsequent backcrosses was even more pronounced and the shape of the ascospores was different, being narrower and with pointed ends. Hence it appears that both Abbott strains carry a gene or genes resulting in ascospore abortion in certain genetic backgrounds and that Abbott 4 shows differences from Abbott 12 in this respect.

In addition, some of the progeny of $1A \times 25a$ and $(1A \times 25a) \times 1A$ were crossed to the same isolate of *asco*(37402). The crosses were homogeneous for the centromere distance of *asco* (Table 9). However, progeny of Abbott $12a \times 1A$ crossed to the same isolate of *asco* as above gave centromere distances of *asco* which were significantly different (Table 8).

Hence it can be concluded that the presence of Abbott ancestry in crosses is the cause of heterogeneity in centromere distances, that the Abbott strains differ genetically from one another, and that the Lindegren strains of opposite mating-type give homogeneous results.

Finally direct crosses of a specific Abbott with a Lindegren strain give as expected homogeneous results since each ordered tetrad from such a cross reflects directly the similar recombinational events occurring during meiosis in each diploid ascus fusion nucleus. However, in the progeny of such a cross might segregate the factor(s) controlling different centromere distances if these are, in fact, genetically determined. Thus heterogeneous centromere distances might arise among such progeny when these are backcrossed to either parent, e.g. (Abbott 4 × Lindegren) × Abbott 4.

It would appear from the homogeneity of the above backcross data that this is not the case, but if the data are plotted graphically by the method of Mosteller & Turkey (1949) a greater spread is apparent in the cases of the 'backcrosses' compared with the 'direct crosses'. When the centromere distance of *mt*, plus or minus twice its standard error, is plotted for each group of wild-type ancestry (Table 2), the distances fall into three significantly different groups of values (Frost, 1955*b*). This suggests that if centromere distance is genetically determined, it is controlled among the various wild strains by three genetic factors or groups of factors.

5. HETEROGENEITY IN CENTROMERE DISTANCES OF LOCI IN LINKAGE GROUPS I TO VII

(*a*) *Chilton crosses*

The limited data are given in Table 3. The behaviour of the loci listed follows that of *mt* with the corresponding wild-type ancestries. Furthermore, the presence of Chilton as opposed to Lindegren genetic material introduces no further heterogeneity, again suggesting that these two wild strains show similar patterns of recombinational events.

(*b*) *Abbott 12 crosses*

The data are given in Table 4. It can be seen that the behaviour of the loci involved in crosses of Abbott 12 × Lindegren ancestry also follow that of *mt* with

Table 3. *Centromere distance of loci from crosses involving Chilton* a

Data* source	Locus	Link group	Wild-type ancestry†	No. of crosses	2nd-div. asci	Total asci	Het. χ^2	P(%)	Centromere distance	2×2 χ^2	P(%)
1	*thi*(9185)	III	L × L	1	7	16	—	—	21·88 ± 6·20	0·012	90–95
			L × C	1	5	12	—	—	20·83 ± 7·17		
1	*pdx-1*(37803)	IV	L × L	6	37	199	10·175	5–10	9·30 ± 1·33	0·500	30–50
			(A4 × C) × L	2	7	29	2·461‡	10–20	12·07 ± 3·97		
1	*pyr-2*(38502)	IV	L × L	2	30	46	1·304	20–30	32·61 ± 3·51	3·188	5–10
			(A4 × C) × L	1	3	9	—	—	16·67 ± 7·86		
1, 2	*me-3*(36104)	V	L × L	3	33	60	1·212	50–70	27·50 ± 3·21	0·152	50–70
			(A4 × C) × L	1	12	20	—	—	30·00 ± 5·48		
1	*nt*(39401)	VII	L × L	1	11	32	—	—	17·19 ± 4·20	0·279	50–70
			(A4 × C) × L	1	4	15	—	—	13·33 ± 5·71		

* 1. Houlahan *et al.* (1949); 2. Buss (1944).
† A = Abbott; C = Chilton; L = Lindegren.
‡ 2×2 χ^2 test modified for small samples.

Table 4. *Centromere distance of loci from crosses involving Abbott 12*

Data* source	Locus	Link group	Wild-type ancestry†	No. of crosses	2nd-div. asci	Total asci	Het. χ^2	P(%)	Centromere distance	2×2 χ^2	P(%)
1	*arg-6*(29997)	I	L×L	1	20	32	—	—	31·25 ± 4·28	0·111	70–80
			L×A12	1	58	98	—	—	29·59 ± 2·48		
2	*thi*(9185)	III	L×L	1	7	16	—	—	21·88 ± 6·20	0·385	70–80
			L×A12	2	32	61	0·077	70–80	26·23 ± 3·20		
3	*me-5*(9666)	IV	L×L	5	50	87	0·947	90–95	28·74 ± 2·65	3·299	5–10
			L×A12	1	7	20	—	—	17·50 ± 5·33		
2	*pyr-1*(H263)	IV	L×L	2	13	108	0·045	80–90	6·02 ± 1·57	0·209	50–70
			L×A12	1	2	23	—	—	4·35 ± 2·94		
2	*pdx-1*(37803)	IV	L×L	6	37	199	10·157	5–10	9·30 ± 1·33	0·069	70–80
			L×A12	1	4	19	—	—	10·53 ± 4·68		
2	*pab-1*(1633)	V	L×L	1	7	16	—	—	21·88 ± 6·20	5·510	1–2
			L×A12	1	27	35	—	—	38·57 ± 3·55		
3	*me-3*(36105)	V	L×L	5	57	99	13·817	< 1	*c.*28·8	—	—
			L×A12	1	12	20	—	—	30·00 ± 5·48		

* 1. Srb (1946); 2. Houlahan *et al.* (1949); 3. Buss (1944).
† A = Abbott; L = Lindegren.

corresponding ancestry. The only exception is *pab-1*(1633). Of the four crosses listed by Houlahan *et al.* (1949), one involves a translocation and another is of mixed Lindegren and Abbott 4 ancestry and so both have been rejected. The remaining two crosses are heterogeneous, and when the four-fold χ^2 test modified for small samples is used then $\chi^2 = 4{\cdot}110$, $n = 1$, $P = 0{\cdot}02$–$0{\cdot}05$. Thus *pab-1* shows a significantly increased centromere distance in the Abbott 12 × Lindegren cross. With *me-3*(36105) the heterogeneity is present in the crosses of supposed pure Lindegren ancestry.

(*c*) *Emerson 5297 crosses*

The data are given in Table 5. Here the behaviour of some of the loci does not follow that of *mt* which showed a significant increase in centromere distance over the pure Lindegren value in E5297 crosses. In Group III, *thi*(9185) shows a significantly reduced centromere distance. Although Abbott 12 is concerned in the ancestry of E5297, *thi* showed no reduction in centromere distance in a cross to Abbott 12 (Table 4). Again, unlike *mt*, *pyr-2*(38502) shows no increase in centromere distance over the pure Lindegren value. However, the data for *aur* and *inos* come from the same cross, and thus both loci, although in different linkage groups, show simultaneously a significantly increased centromere distance compared with the pure Lindegren value.

This different pattern of heterogeneity does not appear to be associated with distance of the locus from its centromere. Perhaps the E5297 strain carries some chromosomal aberration or possibly if centromere distance is genetically determined the factor(s) concerned may act with different intensities in the different linkage groups.

(*d*) *Abbott 4 crosses*

The data are given in Table 6. Similarly to the case of *mt*, two out of four loci show significant reductions in centromere distance in Abbott 4 × Lindegren crosses as opposed to crosses of pure Lindegren ancestry. In the case of *chol-1* and its allele the Lindegren crosses are heterogeneous but nevertheless the Abbott 4 × Lindegren cross gives a much lower centromere distance. In addition *pab-1* and *nt* behave like *mt* in (Abbott 4 × Lindegren) × Lindegren crosses. In the case of *nt*, two crosses are given by Houlahan *et al.* (1949) but they are heterogeneous. One gave only 65% germination and so may be biased and has been discounted.

However, a tendency emerges, with mixed Abbott 4 and Lindegren ancestries, for loci proximal to their centromere to show a different pattern of heterogeneity in centromere distance from loci distal from their centromere. In Abbott 4 × Lindegren crosses, proximal loci show a significant reduction whereas distal loci do not, while in (Abbott 4 × Lindegren) × Lindegren crosses distal loci tend to show an increase whereas proximal loci do not. The distance from the centromere at which these phenomena take place might vary in the different linkage groups.

The data for *me-3* do not support this hypothesis, but if the four-fold χ^2 test for small samples is used, then $\chi^2 = 3{\cdot}676$, $n = 1$, $P = 0{\cdot}05$–$0{\cdot}10$. Thus a reduction in

Table 5. *Centromere distance of loci from crosses involving E5297*

Locus	Link group	Wild-type ancestry*	No. of crosses	2nd-div. asci	Total asci	Het. χ^2	P(%)	Centromere distance	2×2 χ^2	P(%)
aur(34508)	I	L × L	12	141	270	12·301	30–50	26·11 ± 1·52	20·129	< 1
		[(A4 × L) × L] × E5297	1	93	122	—	—	38·12 ± 1·93		
thi(9185)	III	L × L	1	7	16	—	—	21·88 ± 6·20	4·015	2–5
		L × E5297	1	28	132	—	—	10·61 ± 1·79		
pyr-2(38502)	IV	L × L	2	30	46	1·304	20–30	32·61 ± 3·51	0·164	50–70
		L × E5297	1	12	20	—	—	30·00 ± 5·48		
inos(37401) (83201)	V	L × L	7	66	133	8·472	20–30	24·81 ± 2·17	6·614	1–2
		[(A4 × L) × L] × E5297	1	80	122	—	—	32·79 ± 2·15		

All data from Houlahan *et al.* (1949).
* A = Abbott; E = Emerson; L = Lindegren.

Table 6. *Centromere distance of loci from crosses involving Abbott 4*

Data* source	Locus	Link group	Wild-type ancestry†	No. of crosses	2nd-div. asci	Total asci	Het. χ^2	P(%)	Centromere distance	2×2 χ^2	P(%)
1, 2	*al-1*(4637T)	I	L × L	15	33	298	19·715	10–20	5·54 ± 0·91	8·009	< 1
			L × A4	3	1	85	0·964	50–70	0·59 ± 0·58		
1	*chol-1*(37903) (34542)	IV	L × L	5	74	138	11·116	2–5	*c.* 26·8	—	—
			L × A4	1	5	30	—	—	8·33 ± 3·40		
1	*pyr-2*(38502)	IV	L × L	2	30	46	1·304	20–30	32·61 ± 3·51	0·084	70–80
			(A4 × L) × L	1	7	10	—	—	35·00 ± 7·25		
3	*me-3*(36104)	V	L × L	3	33	60	1·212	50–60	27·50 ± 3·21	4·756	2–5
			L × A4	1	5	19	—	—	13·16 ± 5·05		
1	*inos*(37401)	V	L × L	7	66	133	8·472	20–30	24·81 ± 2·17	0·008	90–95
			L × A4	1	21	43	—	—	24·42 ± 3·81		
1	*pab-1*(1633)	V	L × L	1	7	16	—	—	21·88 ± 6·20	1·706	10–20
			(A4 × L) × L	1	7	10	—	—	35·00 ± 7·25		
1	*nt*(39401)	VII	L × L	1	11	32	—	—	17·19 ± 4·20	1·019	30–50
			L × A4	1	4	19	—	—	10·53 ± 4·68		
1	*nt*(39401)	VII	L × L	1	11	32	—	—	17·19 ± 4·20	3·153	5–10
			(A4 × L) × L	2	15	26	1·348	20–30	28·85 ± 4·84		

* 1. Houlahan *et al.* (1949); 2. Srb (1946); 3. Buss (1944).
† A = Abbott; L = Lindegren.

centromere distance for *me-3* is barely significant. On the other hand, Barratt (1954) reported that Teas (1947) had observed a significantly increased recombination frequency for *aur*(34508) and *al-2*(15300) in crosses with *thr*(44104). These crosses are of (Abbott 4 × Lindegren) × Lindegren ancestry and thus do not behave similarly to *mt* but *aur* and *al-2* are distal from their centromere.

The data (Table 7) for *ad-4*(44206) and *ad-2*(70004t), both in the right arm of linkage group III, also show this effect of distance. The centromere distances from

Table 7. *Centromere distance of* ad-2 *and* ad-4 *both in the right arm of linkage group III from crosses of complex wild ancestries involving Abbott 4*

Locus	Cross* ancestry	No. of crosses	2nd-div. asci	Total asci	Het. χ^2	P(%)	Centromere distance
ad-2(70004)	1	3	19	49	0·564	70–80	19·39 ± 3·48
	2	2	17	32	0·126	70–80	26·56 ± 4·41
	3	2	14	45	3·241	5–10	15·56 ± 3·45
	4	1	8	14	—	—	28·57 ± 6·61
ad-4(44206)	1	7	10	150	12·307	5–10	3·33 ± 1·02
	2	3	16	55	11·916	< 1	c. 14·6
	3	2	6	40	0·373	50–70	7·50 ± 2·82
	4	3	4	56	0·403	80–90	3·57 ± 1·72

All data from Houlahan *et al.* (1949).

* 1. = (A4 × L) × L; 2. = (A4 × L)⊕; 3. = [(A4 × L)⊕] × L; 4. = [(A4 × L) × L] × L; where A = Abbott, L = Lindegren and ⊕ = selfed.

crosses of four different complex wild-type ancestries compared with one another are heterogeneous only for *ad-4* which is nearer the centromere. Furthermore, only this locus shows heterogeneity within crosses of one given ancestry type. The seven crosses of type 1 ancestry are sharply divided into two groups, four crosses giving a centromere distance of 0·0 and three crosses giving a distance of 6·49 ± 1·92. A significant divergence is shown by crosses of type 2 ancestry, one cross giving a distance of 0·0 and the other two, in good agreement with one another, a distance of 22·22 ± 4·14. A possible explanation of this behaviour is that type 1 being 'back-crosses' and type 2 'selfings', segregation of the genetic factor(s) controlling different centromere distances of a locus had occurred.

(*e*) *Conclusions*

When the crosses are arranged according to wild-type ancestry, heterogeneity in centromere distances of loci in all linkage groups for which there is data is eliminated with few exceptions. Chilton crosses behave similarly to Lindegren but both differ from Abbott 4 and Abbott 12 crosses which also differ from one another. In general the loci show a similar pattern to *mt* with each wild ancestry type. However, loci distal from their centromere behave differently from proximal loci in crosses involving Abbott 4 and Lindegren ancestries. In addition some variation in the pattern of heterogeneity in centromere distance may occur between linkage groups.

Finally, segregation of the genetic factor(s) controlling heterogeneity in centromere distances of a locus appears possible.

6. SEGREGATION OF THE GENETIC FACTORS CONTROLLING DIFFERENT CENTROMERE DISTANCES OF A LOCUS

To investigate further the possible segregation of one or more factors controlling different centromere distances of a locus and at the same time to obtain data, which were lacking, on Abbott 12 × Lindegren backcrosses, Abbott 12*a* was crossed with Lindegren 1*A*. Ascus 9 showing first-division segregation for *mt* was chosen and cultures of spore pair 1 and pair 2 were each backcrossed to the Abbott parent, and pair 3 and pair 4 to the Lindegren parent. The data for the resulting centromere distances of *mt* are given in Table 8.

Comparatively immature, as well as mature, asci were dissected by the method described above so that any bias due to second-division asci maturing first would be minimized. The crosses with identical strains were repeated several times and consistent results were obtained, homogeneity of the crosses for each spore pair were all in the probability levels between 70 and 95%. The total sample sizes were increased to reduce errors due to small sample sizes. Records were kept of the number of non-germinating spore pairs and the number of asci which could not be scored for *mt*. These showed that high or low centromere distances resulted both with complete germination and analysis or with some failures. Hence viability differences were not causing bias.

The results (Table 8) indicate that there is a segregation in the ascus of one or more factors controlling significantly different centromere distances of *mt*, as if centromere distances were genetically determined (Frost, 1955*b*).

Table 8. *Segregation of heterogeneous centromere distances of* mt *and* asco *in Ascus 9 arising from the cross Abbott 12*a × *Lindegren 1*A

Ascus 9 spore pair	2nd-div. asci	Total asci	Asci dissected	Centromere distance	2×2 χ^2	P(%)
		× Abbott 12*a*. Scored for *mt*				
1*A*	8	57	64	7·02 ± 2·30	4·299	2–5
2*A*	27	94	102	14·37 ± 2·33		
		× Lindegren 1*A*. Scored for *mt*				
3*a*	18	117	117	7·69 ± 1·67	4·939	2–5
4*a*	20	69	79	14·49 ± 2·73		
		× *asco*(37402)*a*. Scored for *asco*				
1*A*	81	347	—	11·67 ± 1·14	8·840	< 1
2*A*	121	362	—	16·72 ± 1·24		

Furthermore, cultures of Ascus 9 spore pair 1 and pair 2 were each crossed to the same isolate of *asco* which is in linkage group VI. A segregation of the genetic factor(s) controlling significantly different centromere distances occurred also for

asco. The spore pair which gave the significantly lower distance for *mt* also gave the significantly lower distance for *asco* and vice versa. Hence two loci in different linkage groups show the same pattern of heterogeneity.

However, the same isolate of *asco* was crossed to some random spore progeny of pure Lindegren crosses, viz. $1A \times 25a$ and the backcross $(1A \times 25a) \times 1A$. Here the centromere distances of *asco* are homogeneous (Table 9). Comparing these data with

Table 9. *Centromere distance of* asco(*37402*)a *in crosses to Lindegren 1*A *and to random spores from 1*A × *25*a *and* (*1*A × *25*a) × *1*A

Cross* ×	2nd-div. asci	Total asci	Centromere distance
L1	117	318	18·4
L1/L25–RS2	39	103	18·9
L1/L25–RS3	68	186	18·3
L1/L25–RS6	39	108	18·1
L1/L25–RS7	46	142	16·2
L1/L25–RS8	22	49	22·4
L1/L25/L1–RS3	45	130	17·3
L1/L25/L1–RS5	22	57	19·3
L1/L25/L1–RS6	33	83	19·9
L1/L25/L1–RS9	30	91	16·5
Total	461	1267	

Het. $\chi^2 = 3{\cdot}802$, $n = 9$, $P = 0{\cdot}90$–$0{\cdot}95$.
Centromere distance of *asco* = $18{\cdot}19 \pm 0{\cdot}68$.
* L1 = Lindegren 1*A*; L25 = Lindegren 25*a*; RS = random spore.

the Ascus 9 × *asco* crosses in Table 8, then spore pair 1 × *asco* shows a significant reduction over the Lindegren $1A \times$ *asco* distance ($\chi^2 = 14{\cdot}355$, $n = 1$, $P = < 0{\cdot}01$) but spore pair 2 × *asco* is not significantly different from the $1A \times$ *asco* distance. Unfortunately the wild ancestry of the *asco* strain used is unknown but presumably it is a re-isolate and hence the wild ancestry of these two crosses may be complex.

Combining the heterogeneous data for *mt* from spore pairs 1 and 2 which are crosses of (Abbott 12 × Lindegren) × Abbott 12 ancestry, a centromere distance of 11·3 for *mt* is obtained. This is in good agreement with the data (Table 2) for (Abbott 4 × Lindegren) × Abbott 4 crosses but perhaps through small sample sizes heterogeneity cannot be detected by the χ^2 test in the latter data but the graphical method of Mosteller and Turkey indicates some scatter.

Since E5297 arose from a random spore, it can be compared to spore pair 4 (Table 8) where the cross is of (Abbott 12 × Lindegren) × Lindegren ancestry. The centromere distance of *mt* here is in good agreement with that from E5297 × Lindegren crosses (Table 2) which are of identical wild ancestry. This suggests that segregation of genetic factor(s) controlling different centromere distances occurred in the spore which gave rise to E5297.

Since heterogeneity arises in the centromere distances of the same isolate of *asco* only when Abbott 12 is involved in the ancestry, it may be concluded that Abbott 12 is causing this effect. Although the analyses have been carried out only on one

ascus, it is apparent that at least one genetic factor must be carried by Abbott 12 resulting in a significantly increased centromere distance of *mt*. Furthermore, the comparison with the corresponding Abbott 4 data suggests that Abbott 4 too carries a similar factor or factors. Whether these factors in the two Abbott strains are identical is not apparent.

7. HETEROGENEITY IN RECOMBINATION FREQUENCIES BASED ON RANDOM SPORE ISOLATES

In random spore analyses, Frost (1955*b*) found several cases of heterogeneity in recombination frequencies of which two will be reported here. A three-point test-cross of re-isolated strains of *arg-3* and *ad-3* gave in repulsion 3·2% recombination (3/93) between *arg* and *mt*, but one of the double mutants outcrossed to Lindegren I gave in coupling 15·8% recombination (12/76) in this interval. A comparison gave $2 \times 2\chi^2 = 8{\cdot}162$, $n = 1$, $P = < 0{\cdot}01$. The other intervals were in good agreement. Germination was 76 and 78% respectively and progeny segregations were in good agreement with expectation. From the repulsion cross, 52 ordered asci (germination 78%) gave the order *mt*, *arg-3*, centromere, *ad-3*. However, an isolate of *arg-3* related to that used above was crossed to *al-2*. 43·3% recombination (45/104) occurred in the *arg* to *al* interval, 10·6% (11/104) between *arg* and *mt*, but 40·4% (42/104) between *al* and *mt*, thereby indicating a reversal in the order of *mt* and *arg* as if an inversion were involved. Germination was 77% and progeny segregations in agreement with expectation.

A second case involved a cross of *arg-6* with *nic-1* giving 5·6% recombination (11/197). Germination was 98%. A double-mutant from this cross was then crossed to *al-1* and 12·5% recombination (17/136) occurred between *arg* and *nic*. Germination was 85%. In both crosses progeny segregations were in agreement with expectation. The two recombination frequencies were significantly different ($\chi^2 = 4{\cdot}984$, $n = 1$, $P = 0{\cdot}02$–$0{\cdot}05$).

All the loci mentioned above are in linkage group I. Heterogeneity in centromere distances of loci distal from their centromere could arise by altered recombination frequencies proximal to the centromere only (Rifaat, 1958). These random spore analyses, however, indicate that heterogeneity can be present in recombination frequencies over short intervals distal from as well as proximal to the centromere.

De Serres (1958*a*) found three significantly different groups of recombination frequencies between heteroalleles at the *ad-3* locus in crosses all involving only one wild ancestry, namely 74*A*. As shown in Table 1, however, 74*A* is a hybrid involving Abbott 4, Abbott 12 and Lindegren 1 and 25. It is of interest that three significantly different groups of values were obtained by De Serres as in the case of the centromere distances of *mt* reported above here where the same mixed wild ancestries are involved.

8. HETEROGENEOUS RECOMBINATION FREQUENCIES IN OTHER FUNGI

Heterogeneous recombination frequencies do not appear to be confined to *Neurospora crassa*. Fincham (1951) pointed out that the Arlington strain of *N. sitophila*

gave a lower centromere distance of *mt* than the English strain of this species. In addition, the data of Wülker (1935), which were derived from the second and third backcrosses to *N. sitophila* of an original cross of a strain of *N. sitophila* with a strain of *N. crassa*, gave a significantly reduced centromere distance of *mt* compared with the value from the English strain of *N. sitophila*.

Papazian (1950, 1951) found that when three wild strains from different localities of *Schizophyllum commune* were inbred, in two of the crosses recombination occurred in the *A* mating-type locus but the recombination frequency in these two crosses between *A* and *s* (streak) was significantly lower than in the remaining crosses in which no recombination occurred in the *A* locus. Here then an alteration occurred in both the frequency and distribution of recombination.

Dr J. R. Raper (personal communication) and Raper, Baxter & Middleton (1958) claimed that the frequencies of the origin of new mating-type alleles by recombination in their stocks of *Schizophyllum commune* showed significant differences between crosses involving different wild strains. The greatest divergence occurred when a wild strain from California and another from New Guinea were crossed with local wild strains from Illinois.

Day (1958) found heterogeneity in recombination frequencies in *Coprinus lagopus*. He suggested this might have arisen from the use of different wild strains in the crosses concerned.

In *Venturia inaequalis*, Boone & Keitt (1956) reported discrepancies in centromere distances and the occurrence of extra-chromosome progeny similar to pseudo-wild-types in *Neurospora*. Here again different wild strains may have been involved in the ancestry of the crosses.

In contrast all the mutant strains of the homothallic fungus *Aspergillus nidulans* arose or have been induced from a single wild-type strain (Pontecorvo, 1953). The extensive data obtained from these strains have revealed no case of heterogeneity in recombination frequencies.

In addition Whitehouse (1954), using strains of *Coprinus fimetarius* all of which were descended from a single wild fruit-body, failed to detect recombination in the incompatibility loci.

Frost (1955*b*) suggested that the use of mixed wild ancestries instead of one might be experimentally convenient in significantly increasing and so detecting rare recombination between pseudo-alleles. The work of De Serres (1958*a*) supports but does not conclusively prove this suggestion since in his crosses involving only one wild-type ancestry he used 74*A* which is a hybrid of several wild strains.

9. DISCUSSION

The experiments and data given above do not support the suggestions made by a number of workers that heterogeneity in recombination frequencies might arise from early maturation or preferential germination of certain asci or random spores; or from the presence of chromosome aberrations; or from irradiation effects additional to the induction of the biochemical mutation. The latter explanation

might be tenable for the exceptional behaviour of strain 27663 which gave significantly different centromere distances when two crosses both of Abbott 4 × Lindegren ancestry were compared and when opposite mating-types of 27663 were crossed to *ad-4*(44206) (data from Houlahan *et al.*, 1949).

On the other hand, the work of Singleton (1948), Tatum & Bell (1946), Holloway (1953), Garnjobst (1953), Hirsch (1954), Faull (1930) and Fincham (1950, 1951) indicates that the different wild strains of *N. crassa* show cytogenetical, physiological and ecological differences. In general these differences are of the same order, but not of the same magnitude, as the differences between *N. sitophila* and *N. crassa*. Thus compared to one another the wild strains of *N. crassa* are at least genically different or even carrying chromosome complements which are no longer strictly homologous.

Hence the genetical factors controlling recombination frequency might be the distinct genomes of Abbott 4, Abbott 12 and Lindegren wild-types. Since heterogeneity in recombination frequency can arise between heteroalleles, a locus and its centromere, or within short marked intervals proximal or distal to a centromere, the simplest explanation would be an alteration in effective pairing. Pontecorvo (1959) has suggested that heterozygocity might increase the frequency of exchanges in effectively paired segments. Thus the reduced frequencies in Abbott 4 × Lindegren crosses could be attributed to a lack of homology. As shown above, all other crosses of mixed Abbott and Lindegren ancestry result in unchanged or increased frequencies. However, in the first backcross to Abbott 4 of an Abbott 4 × Lindegren hybrid not only is homology restored but recombination frequencies may be increased. According to Perkins (1959), Stadler found a tendency for increased frequencies with successive backcrosses, i.e. homozygocity.

It would appear more likely, as Frost (1955*a*) suggested, that the Abbott strains are roughly intermediate between the Lindegren strains of *N. crassa* and the New Zealand strains which are more closely allied to *N. sitophila* (Fincham, 1950). In his studies on the comparative maps of the mating-type chromosome of *N. crassa* and *N. sitophila*, Fincham (1951) stated that much of the difference could be explained by the degree to which the centromere interfered with crossing-over in its vicinity.

This interference could be strong in the Lindegren strains but far less so in the Abbott wild-types and might be under genetic control. The diploid ascus fusion nucleus of Abbott 4 and Lindegren might then intensify this interference for proximal loci. The backcrosses to Abbott 4 would show segregation of strong centromeric interference but also even increased recombination frequencies as shown for *ad-4* above. Possibly the genetic control could reside in the centromere itself.

However, centromeric interference could not explain all the differences since distal linked loci also show increased recombination frequencies. The Abbott strains must also carry genetic factors increasing detectable exchanges in effectively paired segments over the whole length of the chromosomes. It appears that these patterns of recombination are genetically controlled simultaneously in all the linkage groups but possibly with some variation between groups.

Such a hypothesis does not disagree with the claim by Stadler (1956*a*) that many separable hereditary factors affected crossover frequency, or the claim of Rifaat (1958) that at least two genes with equal and cumulative effect controlled recombination frequencies in small regions adjacent to the centromere in linkage group I.

Barratt (1954) cautioned workers on the possible effects that Abbott stocks might have on mapping, interference and pseudo-allelic data. As far as interference studies are concerned, Howe (1956), Stadler (1956*b*) and Perkins (1959) obtained results in conflict with those of Lindegren (as re-analysed by Whitehouse, 1948). It should be pointed out that the former workers used strains of mixed Abbott and Lindegren ancestry, unlike Lindegren, who used only the pure Lindegren wild and mutant strains. On the hypothesis above by which heterogeneity might arise, this conflict between the two sets of data is not unexpected.

Barratt (1954) suggested minimizing the effects of Abbott stocks by an intensive inbreeding programme. With several factors controlling recombination frequency this may not be effective. As shown above, the Lindegren wild strains give consistent, homogeneous results. Thus heterogeneity in recombination frequencies could be eliminated by repeatedly backcrossing all markers used to the Lindegren wild-type (Frost, 1955*b*). This and other phenomena reported above are being investigated further.

SUMMARY

The available data on heterogeneity in centromere distances for a number of loci in several linkage groups are analysed and interpreted. When the crosses are grouped according to wild-type ancestry, heterogeneity is eliminated in any one group except those which consist of backcrosses or intercrosses. Abbott 4 and 12 are shown to be the source of the heterogeneity while Lindegren and probably Chilton wild strains give consistent, homogeneous distances. In a cross between Abbott 12 and Lindegren wild-types, the centromere distances of *mt* and *asco* show heterogeneous values between the spore pairs in an ascus indicating that significantly different distances are genetically determined and that the factors concerned show segregation. The genetic determination differs in the various wild strains; the data suggest that at least three factors are involved. In random spore analyses heterogeneity is present in recombination frequencies between linked markers either proximal or distal to their centromere. The mechanism by which heterogeneity in the data might arise is discussed. To obtain homogeneous data it is suggested that all markers used should be repeatedly backcrossed to the Lindegren wild-type.

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Sensitivity of the *Drosophila* testis to tri-ethylene melamine (TEM)

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When *Drosophila* males are treated mutagenically, different stages of spermatogenesis respond to different degrees. This is true for all mutagens which so far have been tested from this point of view. TEM is one of the most effective mutagens and a potent carcinostatic agent. Its differential action on the male germ-cell stages of *Drosophila* was studied by Fahmy & Fahmy (1955). They came to the conclusion that the highest frequency of recessive lethal and visible mutations is produced in spermatids, probably at the onset of nuclear condensation. Cattanach (1959) scored dominant lethals and translocations in the progeny of male mice that had been injected with TEM. He found that both effects are most frequent in spermatozoa that are utilized from the tenth to the fourteenth day after treatment and presumably had been treated as spermatids. Since X-rays, too, produce their greatest mutagenic effect on spermatids of *Drosophila* (Lüning, 1952; Auerbach, 1954) and mice (Auerbach & Slizynski, 1956), these findings seemed to put TEM into the same class as X-rays as far as differential effects on male germ-cell stages are concerned. Several discrepancies, however, remained to be explained. Firstly, Fahmy & Fahmy (1955) reported that after treatment with TEM Minutes and chromosome fragmentations have their peak sensitivity at a later stage than recessive mutations; but, as shown elsewhere (Auerbach & Sonbati, 1960), this claim is not borne out by the published data. Secondly, also in experiments on *Drosophila* by Fahmy & Fahmy (1954), TEM-induced dominant lethals were found to be most frequent in mature spermatozoa, in contrast to recessive lethals and in contrast to dominant lethals in the mouse. In view of the great difficulties involved in the scoring of dominant lethals in *Drosophila*, it was felt that the mice data are probably more reliable in this regard. Thirdly, Auerbach & Sonbati (1960) found that mustard gas has its strongest mutagenic effect on late spermatogonia of *Drosophila*. This was of interest, since it seemed to point to a difference in action between two closely related chemical mutagens, TEM and mustard gas. The methods of analysis had, however, been different for these two substances. Auerbach & Sonbati analysed sensitivity to mustard gas genetically, using induced crossovers as landmarks for assigning germ-cell stages to successive broods. This method makes use of a dual-purpose stock which allows the scoring of lethals and induced crossovers in the progeny of the same treated males. The latest stage during spermatogenesis when

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crossing-over can be induced is obviously prophase of meiosis. Broods containing induced crossovers must therefore trace back to treated meiotic or premeiotic germ-cell stages, although in the first such brood an admixture of post-meiotically treated germ-cells is possible. In later broods, crossing-over in spermatogonia results in bunches of identical or complementary crossovers. Fahmy & Fahmy (1955) analysed sensitivity to TEM in a different way. They compared the brood pattern of sex-linked lethals with the cytological picture of the testis in males treated with the same dose of TEM; interpretation by this method relies to a considerable extent on assumptions about rate of sperm utilization and amounts of viable spermatozoa formed from different germ-cell stages. It was therefore decided to repeat the brood pattern analysis for TEM by the method used for X-rays and mustard gas.

MATERIAL AND METHODS

One-day-old ♂♂ of the genotype *y* sc^{S1} *In49* sc^{S}; $\frac{dp\ b\ cn\ bw}{+\ +\ +\ +}$ were injected with 0·2 μl. of TEM at a concentration of 2×10^{-4} M in 0·4% saline. Mortality was between 40 and 50%. Twenty-four hours after injection, the ♂♂ were mated individually to three virgin ♀♀ from a strain homozygous for *dp b cn bw* (dumpy, black, cinnabar, brown; second chromosome). At intervals of two or three days they were remated to three new ♀♀, and the old ♀♀ were allowed to produce more progeny in a second vial. Crossovers in F_1 and sex-linked lethals in F_2 were scored separately for each brood and each ♂. Only ♂♂ that had given offspring in all broods were used for the test. Care was taken to collect F_1 ♀♀ for lethal tests from among early as well as late hatchers, since Yanders (1958) has shown that late hatchers have a higher proportion of heterozygotes for a lethal than early hatchers.

RESULTS

Two experiments were carried out, and their results are presented in Tables 1 and 2, and in Fig. 1. In both experiments, progeny was sampled over a period of 12 days, that is from the second to the thirteenth day after treatment. In Experiment 1, this period was subdivided into four broods of 3 days each. In Experiment 2, it was subdivided into five broods, lasting successively 2, 2, 3, 3 and 2 days. In both experiments, the peak of mutation frequency was reached in the third brood. Thus in Experiment 2, two broods of 2 days each had exhausted the store of spermatozoa that had been treated at post-sensitive stages, while in Experiment 1 one brood of 3 days had been insufficient for this purpose. The third broods of the two experiments overlap on the eighth day; it is likely that with daily changes of ♀♀ the highest mutation frequency would have been reached at or around this day. In both experiments, mutation frequency was higher in the second than in the first brood; this may be the expression of a tendency for sensitivity to decrease gradually from the sensitive stage to mature sperm. In both experiments, mutation frequency at the end of the 12-day breeding period was lower than in mature sperm, but this low level was reached abruptly in Experiment 1 and gradually in Experiment 2.

Apparently, spermatozoa treated during the sensitive stage had been used up after 9 days in Experiment 1, but not yet after 7 days in Experiment 2. It may be concluded that under the conditions of these experiments, mutation frequency increased with time available for mating, reached a high level between the sixth and tenth day after treatment, possibly with a peak on the eighth day, and on the eleventh day fell to a level below that in mature sperm.

The crossing-over data can be used for interpreting this temporal pattern in terms of sensitivity differences between stages of spermatogenesis. Only flies in which two marker genes were uncovered can be confidently scored as crossovers. Uncovering of single markers may be caused by mutations or deficiencies; the latter

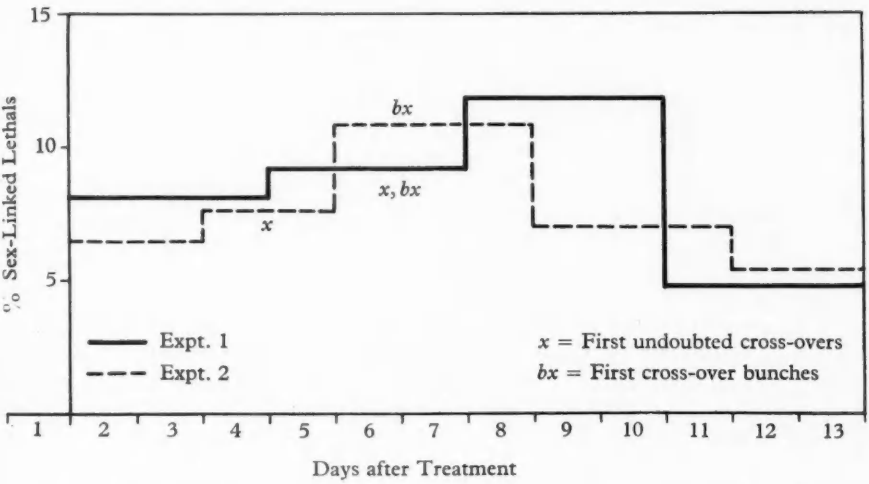

Fig. 1. The brood pattern for sex-linked lethals after treatment of adult males with TEM.

are known to be frequent after treatment with TEM (Fahmy & Fahmy, 1953). Simultaneous uncovering of three genes may happen rarely through reverse mutation or suppressor mutation.

Experiment 1 (Table 1)

None of the exceptional flies in the first brood can with certainty be attributed to crossing-over. The seven *dp* flies may have arisen through mutation or deficiency. The single *dp b cn* fly is more difficult to explain. It may have been due to reverse mutation or suppressor mutation; alternatively, it may have arisen through crossing-over in one of the first treated spermatocytes to form mature sperm. The occurrence of crossovers in the second brood is beyond doubt; three of them occurred as a bunch of two identical and one complementary types. These may trace back to a treated spermatogonium, but this is not necessarily true. Apart from the consideration that crossovers within the same chromosome region may

Table 1. *Sex-linked lethals and apparent crossovers in successive broods from treated males*

Experiment 1

Brood ...	A			B			C			D		
Days ...	3			3			3			3		
Crossover data	N	X		N	X		N	X		N	X	
	3541	7 *dp* 1 *dpb cn*		3057	1 *dp* 1 *cn bw* 1 *dp b* 2 *dp b* 1 *cn bw*		2477	2 *bw* 1 *cn* 2 *dp* 2 *cn* 1 *dp* 1 *bw*	2 *dp* 2 *cn* 1 *cn bw* 1 *bw*	1980	1 *bw* 2 *cn* 1 *dp* 1 *dp b cn*	
Sex-linked recessive lethals	*n*	*l*	%	*n*	*l*	%	*n*	*l*	%	*n*	*l*	%
	466	38	8·1	348	32	9·2	464	55	11·8	315	15	4·76

N Non-crossovers.
X Apparent crossovers (including mutations or deficiencies at the marker loci).
n Number of X chromosomes scored.
l Number of lethals.
Exceptional flies bracketed together came from the same male; all other exceptional flies came from different males.

arise by chance coincidence, Parker (1948) has provided evidence for the suggestion that spurious crossover bunches may be formed in synchronously dividing spermatocytes. Independent of whether the crossovers in the second brood came from treated spermatocytes of spermatogonia, it is clear that the following, third, brood was not produced from post-meiotically treated germ-cells. It is in this brood that the peak

Table 2. *Sex-linked lethals and apparent crossovers in successive broods from treated males*

Experiment 2

Brood ...	A			B			C			D			E		
Days ...	2			2			3			3			2		
Crossover data	N	X		N	X		N	X		N	X		N	X	
	3222	0		3006	2 *cn bw*		2907	2 *cn bw* 1 *cn bw* 2 *dp b*		2033	2 *dp* 1 *cn bw* 3 *dp b cn*		2326	1 *dp* 1 *bw*	
Sex-linked recessive lethals	*n*	*l*	%	*n*	*l*	%	*n*	*l*	%	*n*	*l*	%	*n*	*l*	%
	600	39	6·5	720	55	7·6	760	82	10·8	800	56	7·0	800	42	5·4

N Non-crossovers.
X Apparent cross-overs (including mutations or deficiencies at the marker loci).
n Number of X chromosomes scored.
l Number of lethals.
Exceptional flies bracketed together came from the same male; all other exceptional flies came from different males.

of mutation frequency was found. Thus, the most sensitive stage to TEM in this experiment occurred in late spermatogonia or early spermatocytes, more probably in the former. Younger spermatogonia, represented in the fourth brood, had already passed the sensitive stage.

Experiment 2 (Table 2)

The results of this experiment confirm the conclusions drawn from the first. Again, the first undoubted crossovers occurred in the brood preceding that with the highest mutation frequency, while relatively few mutations were found in the last brood.

DISCUSSION

The interpretation of brood patterns by the method used here is not free from ambiguities. The first brood in which crossovers occur may still contain progeny from germ-cells treated after meiosis. Bunches may be attributed to treated spermatocytes or spermatogonia. It is, therefore, not possible to decide from the evidence presented above whether the sensitive stage of the *Drosophila* testis to TEM occurs in early spermatocytes or late spermatogonia or both, although it is more likely to occur in the latter. In any case it is certain that in these experiments the sensitive stage was not post-meiotic, for in both of them the first undoubted crossovers were found in the brood *preceding* the peak of mutation frequency.

This is in contrast to the claim by Fahmy & Fahmy (1955) that peak sensitivity to TEM occurs in late spermatids. Actually, the observational data are the same in both cases; for in the experiments by the Fahmys, as well as in those reported here, the highest mutation frequency occurred in the third brood, which the Fahmys assume to have been drawn from treated post-meiotic germ-cells. Although it is not impossible that differences between strains (Strømnaes, 1959) or experimental conditions may affect the sensitivity pattern, the discrepancy is more easily explained by the difference between the genetical method of 'landmarks' used in the present experiments and the much more inferential and speculative method of correlating genetical and cytological evidence, used by the Fahmys. In fact, where Fahmy & Fahmy mention genetical 'landmarks' in support of their own interpretation (1960), these agree much better with the one presented here. In support of their claim that the first four broods trace back to treated post-meiotic stages, they adduce the observation that the first clusters of visible mutations were found in the fifth brood. Since only large clusters are likely to be detected in Muller-5 tests, in which the tested progeny per male per brood is small, this finding indicates that at least some of the spermatozoa utilized for the fifth brood were treated in early spermatogonial stages. This is confirmed by the further statement that germinal selection became first apparent in the fifth brood; for there are good reasons for believing that germinal selection acts mainly or only on young spermatogonia which have not yet reached the stage of synchronous division within cysts (Pontecorvo, 1944; Auerbach & Moser, 1953; Auerbach, 1954; Auerbach, unpublished). If, then, the fifth brood already contained spermatozoa that had been treated as early spermatogonia, the third brood may well have been derived from treated

spermatocytes or late spermatogonia. It is therefore probable that also in the experiments by Fahmy & Fahmy, TEM (1955) and other mutagens (1960) that produced peaks of mutation frequency in the third brood did do by virtue of a preferential effect on meiotic or late pre-meiotic stages.

While the present results remove a suspected difference between TEM and mustard gas, they reveal an unsuspected one between the testes of *Drosophila* and the mouse. Although the exact location of the most sensitive stage to TEM in mouse spermatogenesis is still under debate (Cattanach, 1959; Bateman, 1960), there can be little doubt that it occurs after meiosis and before the formation of fully mature sperm. We are still completely ignorant of the causes that determine the differential response of germ-cells to mutagens. Almost certainly many factors are involved, including penetration, general toxicity, oxygenation and other changes in the biochemical pattern of the affected cells, and these may act at various levels of the process of mutagenesis. It is therefore hardly surprising that the sensitivity pattern can differ between species. That it can also be affected by slight differences in mutagenic treatment has recently been shown by Ives (1960), who found that in *Drosophila* males peak sensitivity to gamma-radiation from a cobalt source occurs at a somewhat earlier stage than peak sensitivity to X-rays.

In spite of these minor variations, X-rays, gamma-rays, TEM, mustard gas, and several other alkylating agents studied by Fahmy & Fahmy (1958, 1960) resemble each other in the broad outlines of their differential effects on male germ-cells. All these mutagens are effective throughout spermatogenesis; early spermatogonia are least affected; mature spermatozoa are more sensitive than early spermatogonia; and peak sensitivity occurs at some intermediate stage: in spermatids, spermatocytes, or late spermatogonia. Certain other mutagens, including urethane (Vogt, 1950), formaldehyde used as injection (Sobels, 1956) and ethyl methane sulphonate (Fahmy & Fahmy, 1957) act most strongly on mature spermatozoa. Whether the difference between the two groups of mutagens is due to some essential difference of action is doubtful. There appears to be more justification for suspecting that mutagens that, like formaldehyde food (Auerbach & Moser, 1953) and chloroethyl methane sulphonate (Fahmy & Fahmy, 1957), act exclusively or almost exclusively on pre-meiotic stages may do so by virtue of some special interaction with the synthetic processes going on during these stages. For formaldehyde food, this assumption finds support in Alderson's observation (1960) that the presence of ribonucleic acid is a necessary condition for the production of mutations.

SUMMARY

The sensitivity pattern of the *Drosophila* testis to TEM was analysed by means of a dual-purpose strain that allows the scoring of induced crossovers and sex-linked lethals in the progeny of the same flies. It was found that TEM produces the highest frequency of mutations in spermatocytes or late spermatogonia, while early spermatogonia are even less sensitive than mature spermatozoa. The discrepancy between this conclusion and that obtained by Fahmy & Fahmy (1955) is attributed to a difference in the method of analysis. The sensitivity pattern of the *Drosophila*

testis to TEM resembles that to mustard gas and differs from that to X-rays. The sensitivity pattern of the mouse testis to TEM differs from that of the *Drosophila* testis.

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Asymmetrical response to selection for rate of development in *Drosophila subobscura*

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1. INTRODUCTION

It is a common experience in selection experiments to find that the rates of response in the two directions are unequal. This phenomenon is observed in an extreme form in 'plateaued' populations, which, although they have ceased to respond to selection in a forward direction, respond readily to back-selection.

In this paper, an attempt is made to elucidate the genetic situation responsible for the asymmetrical response of *Drosophila subobscura* populations to selection for fast and for slow development. This is a character which in this species appears to have reached a 'plateau' under the influence of natural selection. Earlier work (Maynard Smith & Maynard Smith, 1954; Hollingsworth & Maynard Smith, 1955; Clarke, unpublished) has shown that genetic heterozygotes develop faster than homozygotes, and that it is difficult to increase development rate by selection, although the rate can readily be reduced by inbreeding.

Selection for fast and for slow development has been practised without inbreeding. In order to create conditions which might favour progress towards faster development, we started from a population formed by crossing a number of geographical populations, and selected on a diet with an unusually high level of protein. Selection was also practised on a very low protein diet, but irregular fluctuations in development rate occurred from generation to generation. Their presence made it impossible to detect the relatively small changes to be expected from selection, and therefore the line was discontinued. Despite the unusual environment and hybrid foundation population, very little progress was made towards faster development. But a comparison of this experiment with the earlier experiments by Hollingsworth & Maynard Smith (1955) has made it possible to give a picture of the genetics of development rate in this species which, although complex, may prove to be typical of characters which have in the past been exposed to directional selection for an appreciable time in a reasonably large population.

2. MATERIALS AND METHODS

A geographically hybrid foundation population was obtained by crossing together four wild-type stocks in such a way that each contributed equally to the foundation population. Of the four stocks, two were derived from groups of wild females caught in Edinburgh, Scotland and in Galilee, Israel, respectively, and two were inbred

lines, the structurally homozygous **K** line derived from a female caught in Switzerland, and the **M** line derived from a female caught in Edinburgh. From this foundation population, two lines, F and S, were started, selected respectively for fast and for slow development from egg to adult emergence.

To collect eggs, single fertilized females were transferred to a dark medium consisting of agar and molasses with live yeast suspension added, for a number of successive 6-hour periods. Batches of eight eggs laid in a single period were then collected on paper spoons and transferred to vials containing a food medium of agar, molasses and maize meal, with 12% by weight of dried and killed yeast, and with one drop of dilute live yeast suspension added to the surface. Clarke (unpublished) has found that the optimum concentration of dead yeast both for survival and for fast development is about 4%. As mentioned above, an experiment on a low protein diet, with 0·1% of dead yeast, was also started, but there were large fluctuations in development rate from generation to generation and the experiment was discontinued. During the selected generations, two to three cultures of eight eggs each from about twenty-five females of each line were set up. Emergence of adult flies occurs almost entirely in the morning, and so the number of adults emerging was recorded every afternoon, the development time being taken as the interval between 12 a.m. on the day of emergence and the mid-point of the 6-hour interval during which the eggs were known to have been laid. There is little difference between the emergence times of males and of females, and so all results are given for the two sexes combined. Vials in which fewer than four adults emerged were not included in the results.

The experiment was carried out in a room controlled at $20 \pm 0{\cdot}5\%$° C. But even small changes in temperature, and perhaps differences between the food in successive generations, can have large effects on development time. Therefore all calculations have been based, not on the absolute development times, but on the differences between the development times of the selected lines and that of a control population set up synchronously with each selected generation. This control population was obtained by crossing the structurally homozygous **B** and **K** inbred lines, and mating together the F_1 hybrids to obtain ten to fifteen cultures of eight F_2 eggs each. The F_2 rather than the F_1 was chosen as a control because it is much easier to obtain an adequate number of eggs from F_1 females than from inbred females, and because it is only slightly more variable phenotypically.

After setting up the selected lines, further samples of eggs were collected from the parents, to measure the percentage egg hatch; this was found in most generations to be over 90%.

3. RESULTS

A. Heritability estimates on the foundation population

A half-sib analysis of the foundation population was performed by mating a number of males each to two females, the parents being selected at random from the foundation population. Two batches of six eggs each were collected from each female. The analysis of variance and estimates of heritability are shown in Table 1.

Table 1. *Analysis of the foundation population*

	Degrees of freedom	Variance	Components
Within cultures	506	3·18	Q
Between cultures	62	4·82	$Q+zC$
Between dams	31	7·97	$Q+zC+2zD$
Between sires	30	6·94	$Q+zC+2zD+4zS$

Mean number of flies per culture, $z = 5{\cdot}08$

Total heritability, $h^2_{\text{DAM}} = \dfrac{4D}{Q+C+D+S} = +0{\cdot}33$

Additive heritability, $h^2_{\text{SIRE}} = \dfrac{4S}{Q+C+D+S} = -0{\cdot}054$

Since the true value of the additive heritability cannot be negative, a better estimate of the total heritability is obtained by assuming $S = 0$, when $h^2_{\text{TOTAL}} = 0{\cdot}277$.

Note: In this Table, and in Tables 2 and 3, the unit is a 6-hour period, not a day.

The results show that, even with the precautions taken to reduce environmental variance, the total heritability of development time is low (approximately 0·3), and the estimate based on the sires suggests that there is little or no additive genetic variance.

However, it is worth pointing out that heritability estimates based on half-sib analyses are subject to large sampling errors unless very large numbers of parents are used, and that a more accurate estimate of additive heritability can be obtained with the same amount of labour by performing a single generation of selection.

B. Responses to selection

Selection for fast and for slow development was practised for eleven generations. The results, based on the differences between the selected lines and the controls, are shown in Fig. 1. The emergence time of the controls had a mean of 17·4 days, and a range from 16·8 to 18·1 days.

In the figure, the response to selection has been plotted against the cumulative selection differential, as suggested by Falconer (1955). The linear regression lines show realized heritabilities of $+0{\cdot}186 \pm 0{\cdot}031$ in the S line, and $+0{\cdot}063 \pm 0{\cdot}029$ in the F line. In calculating these regression lines, each point has been weighted by the reciprocal of its error variance, which is proportional to $(n_1+n_2)/(n_1 n_2)$, where n_1 and n_2 are the number of flies in the selected line and in the controls respectively.

The main conclusion to be drawn is that more rapid progress was made in the S than in the F line; indeed, progress towards faster development was barely significant statistically.

The negative responses to selection in the eighth, ninth and tenth generations of the S line call for an explanation. During the early development of the eighth generation the temperature control broke down, the temperature rose to 25° C. for a few days, and in consequence there was a heavy mortality, particularly in the S line. The fall in the development time in the eighth generation of the S line, relative

to the controls, probably occurred because there was in this line a higher mortality among the more homozygous and more slowly-developing genotypes. Since relatively few flies emerged in the eighth generation, little selection could be practised on it. There was also a heavy mortality during the development of the ninth generation of the S line, for reasons which are not clear; consequently little selection could be practised on this generation either. But progress towards slow development was resumed in the eleventh generation. The irregular response to selection during the last few generations therefore does not prove that a plateau had been reached

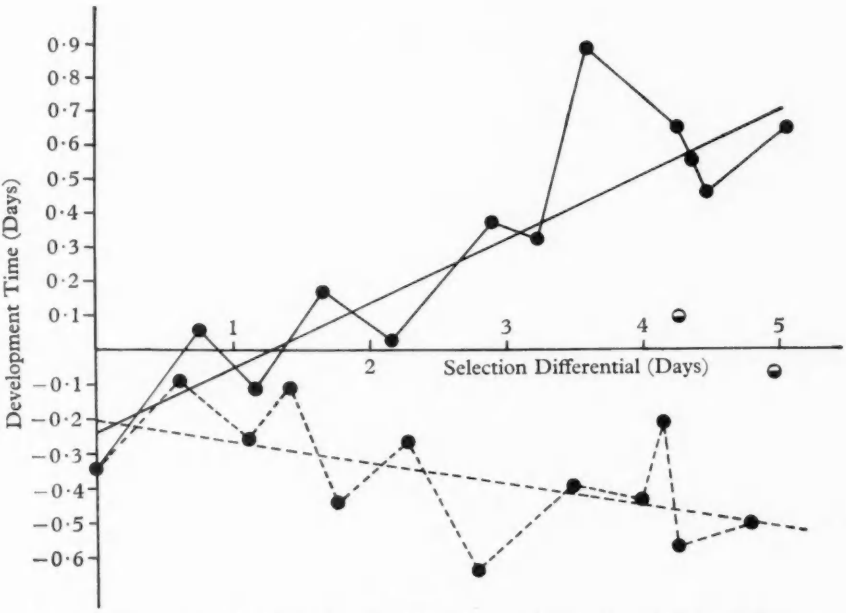

Fig. 1. Response to selection. Full line, S line; broken line, F line. ●, selected lines; ◒, F_1 hybrids between selected lines. The development times are the differences between the measured populations and synchronous **B**/**K** F_2 controls.

beyond which progress could not be made, since the irregularities were at least in part due to the temporary rise in temperature. But the results do suggest that natural selection, particularly when the temperature rose, was counteracting the effects of artificial selection for slower development. It seems likely that if a uniform temperature of 20° C. had been maintained, the value of the realized heritability in the S line would have been somewhat higher than 0·186; but there is no reason to think that the response in the F line would have been any greater.

C. Genetic variance of the selected lines

Analyses of variance of the combined first and second generations, and of the combined tenth and eleventh generations, of the two lines are given in Tables 2 and

Table 2. *Analyses of variance of the combined first and second selected generations*

	Degrees of freedom		Variance		
	F line	S line	F line	S line	Components
Within cultures	407	341	3·09	2·90	Q
Between cultures	34	32	4·16	4·90	$Q+zC$
Between families, within generations	42	30	7·63	8·20	$Q+zC+yF$

	F line	S line
Mean number of flies per culture (z)	6·22	6·32
Mean number of flies per family (y)	11·02	12·64
Total heritability, $h^2 = 2F/(Q+C+F)$	0·176	0·150

3. These show that there was significant genetic variance in both lines at the start of the experiment, but that in the last two generations there was no significant genetic variance in the F line, although the genetic variance of the S line had if anything increased.

Table 3. *Analyses of variance of the combined tenth and eleventh generations*

	Degrees of freedom		Variance		
	F line	S line	F line	S line	Components
Within cultures	544	428	3·15	3·57	Q
Between cultures	61	50	5·00	4·52	$Q+zC$
Between families, within generations	36	29	6·09	14·50	$Q+zC+yF$

	F line	S line
z	6·49	6·29
y	16·92	16·42
h^2	0·037	0·280

D. Crosses between lines

In parallel with the ninth and eleventh generations, the development rates of hybrids between the two lines were measured. The results are given in Table 4. They

Table 4. *Mean development times, in days, of the selected lines and of the F_1 hybrids between them*

Generation	F	S	F♀ × S♂	S♀ × F♂
9	16·81	17·57	17·03	17·20
11	16·56	17·71	17·00	16·99

show that the hybrids were approximately intermediate between the two parental lines, and that there was no significant difference between the reciprocal hybrids.

4. DISCUSSION

The results of the present experiment which call for an explanation are as follows:

(i) The response to selection was asymmetrical; the realized heritabilities were +0·186 in the S line and +0·063 in the F line, the latter being barely significant.

(ii) Hybrids between the F and S lines were approximately intermediate between their parents.

(iii) Analysis of the foundation population suggested that all or most of the genetic variance was non-additive.

(iv) Analysis of the last two generations of the selected populations suggested appreciable genetic variance in the S line, but little or no genetic variance in the F line.

These results can be compared to the results obtained by Hollingsworth & Maynard Smith (1955). This earlier experiment concerned the same character in the same species, but the procedure differed in the following ways:

(i) Selection was combined with brother–sister mating.

(ii) The foundation population consisted of the offspring of a single wild female.

(iii) Selection was carried out on a low protein diet, and less care was taken to standardize environmental conditions.

The results resembled the present ones in their main feature. The response was asymmetrical, considerable progress being made towards slower development but none towards faster development. But there were the following important differences:

(i) The response in the S line was more rapid (4 days after seven generations, instead of 1 day after eleven generations). This was partly due to the fact that the difference between two genotypes is greater on diets with less protein. But this is not the whole explanation. Even on 12% dead yeast the S line obtained in the earlier experiment takes 3·5 days longer to develop than most outbred populations.

(ii) Hybrids between the F and S lines were slightly faster than the F parents.

(iii) There was a striking decline in fertility and viability in both lines: in a replicate experiment from a different wild female, all the S lines were lost due to infertility. There was no comparable decline in fertility in either line in the present experiment.

The results of the earlier experiment can be explained if it is assumed that all the genetic variance of development rate is due to genes with heterotic effects, heterozygotes developing fast and homozygotes slowly. This assumption was confirmed by the observation that the S line was structurally homozygous, whereas the F line continued to segregate for inversions on three of the four long autosomes.

But it is far from certain that the present experiment is to be explained by a similar assumption. There are in fact two types of genetic situation which can give rise to asymmetrical responses to selection; first, non-additive interactions between alleles at the same locus (dominance and overdominance), and second, interactions between alleles at different loci (epistasis). For reasons which will now be discussed, the latter seems the more likely explanation in the present case.

Considering first dominance interactions at single loci, if the gene frequencies are such as to maximize the value of the character being measured, then the genetic variance as estimated by a half-sib analysis will be entirely non-additive, and there will be no response to selection in either direction, unless selection is combined with inbreeding, in which case progress will be made in a downward direction only; this is the hypothesis invoked to explain the earlier experiment by Hollingsworth & Maynard Smith (1955). But if the gene frequencies are not such as to maximize the value of the selected character, a half-sib analysis should reveal some additive genetic variance, and progress should be made in both directions. Yet the rates of progress in the two directions may in some cases be different, although the mean value of the two realized heritabilities should be approximately equal to the estimated additive heritability.

Such situations have been discussed by Falconer (1953, 1955); they are of two kinds. First, if the frequency of alleles for, say, slow development is much lower than that of alleles for fast development, then there is more room for progress towards slow development, and in a long-term selection experiment the response will be asymmetrical. Such a model seems inappropriate to explain the response of a population formed by mixing four geographical populations in about equal proportions. But asymmetrical responses can arise even if the frequencies of the two alleles at a single locus are approximately equal, due to directional dominance: that is, if alleles for fast development tend to be dominant over genes for slow development, or if the heterozygote is faster than either homozygote, but one homozygote is slower than the other. In either case progress towards slow development will be more rapid than towards fast development.

The degree of asymmetry to be expected from directional dominance will be greater the higher the intensity of selection, the higher the total heritability of the character, and the smaller the number of loci involved. It is easy to show that as the number of independent loci involved tends to infinity, the degree of asymmetry tends to zero. An attempt has been made to find a genetic model which will explain the present experiment in terms of directional dominance. It has been assumed that:

(*a*) Development rate is influenced by genes at 10 loci, segregating independently.

(*b*) For each locus, the effects on development time are as follows:

$$a_1a_1, 1+k;\ a_1a_2, 1;\ a_2a_2, 1+\tfrac{1}{4}k.$$

(*c*) In the foundation population, all gene frequencies are 0·5.

(*d*) The environmental variance remains constant in the selected lines, and is such that the total heritability in the foundation population is 0·25.

(*e*) The selected parents consist of all individuals one standard deviation or more away from the mean; the population is assumed large, so that there is no inbreeding.

In this model, the selection intensity and total heritability have been chosen to agree with those obtaining in the actual experiment, and the other features to give a reasonable agreement with the responses to selection actually observed.

With these assumptions, the additive heritability estimated by a half-sib analysis

of the foundation population would be +0·104. The responses to selection for fast and slow development are shown in Fig. 2. The agreement between Fig. 2 and the responses actually obtained is reasonably good. But the model leads to two pre-

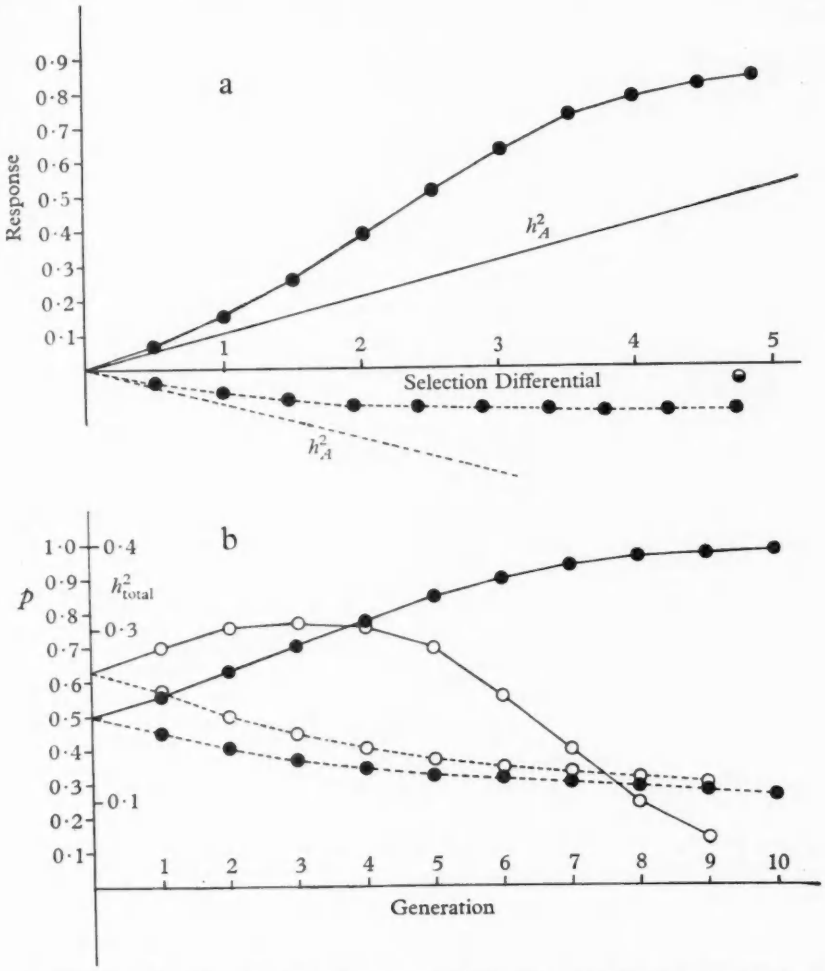

Fig. 2. Theoretical results of selection on a population showing directional dominance. Full lines, S line; broken lines, F line.

a: responses to selection, and responses predicted from a half-sib analysis of the foundation population. ◒, F_1 hybrids between selected lines.

b: ●, frequency p of alleles for slow development; ○, total heritability.

dictions which are contradicted by the experimental results. First, in the model the total heritability would decline to 0·05 in the slow line, but remain reasonably high at 0·12 in the fast line; this is the exact opposite of what actually happened. Second

and more decisive, the model predicts that hybrids between the fast and slow line should be almost as fast as the fast line, whereas in fact the hybrids were roughly intermediate between the two lines. Any attempt to explain the results by a model based on directional dominance would run into the second difficulty. In fact, it seems impossible to devise a model based on interactions between the alleles at single loci which will account adequately for all the features of the present experiment.

It is however possible to suggest a model which will explain the present experiment, if it is assumed that much of the genetic variance is epistatic. Suppose that the

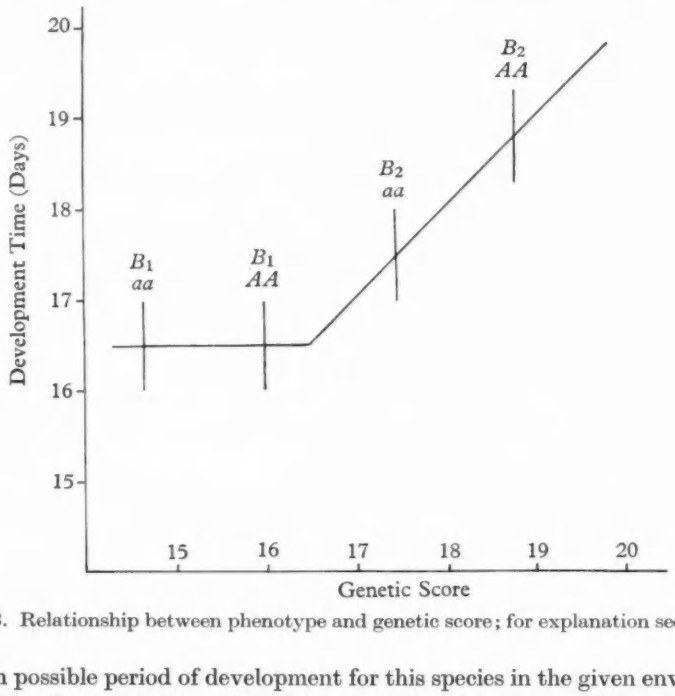

Fig. 3. Relationship between phenotype and genetic score; for explanation see text.

minimum possible period of development for this species in the given environment is 16·5 days. In populations all of whose members take longer than 16·5 days to develop, it is assumed that there is no epistatic genetic variance; i.e. a given gene substitution has the same phenotypic effect whatever the genetic background at other loci. The genetic variance of such a population may be due to genes with additive or with heterotic effects. It would be possible to select from such a population a number of genotypes which, on the assumption of no epistatic variance, would have development times of less than 16·5 days. These development times, predicted on the assumption of no inter-locus interaction, will be called the 'genetic scores' of the individuals. The existence of a developmental barrier at a minimum of 16·5 days implies that all individuals with genetic scores of 16·5 or less will have actual development times of 16·5 days. The relationship between genetic score and phenotype is shown in Fig. 3. The phenotypic effect of a given gene substitution,

say AA for aa, will then be zero on genetic background B_1 (many alleles for fast development) but appreciable on background B (many alleles for slow development).

Situations of this kind, involving a non-linear relationship between genetic score or dosage and phenotype, have been met with in selection experiments on the expression of mutant characters, a partial or absolute barrier existing at the wild-type phenotype (Rendel, 1959; Sondhi, 1960). There is no reason why similar barriers should not influence a character such as development rate, although neither the existence nor the position of the barrier could be predicted *a priori*.

A model of this kind will explain the present results. From previous work, it was to be expected that much of the genetic variance of development time would be heterotic, and consequently that the highly heterozygous foundation population would be close to the minimum possible development time. If so, an appreciable response to selection would be possible only in the S line. But some additive genetic variance would be expected, since geographical populations with different development rates were crossed to obtain the foundation population. Therefore a response would be expected in the S line, although a less rapid one than with inbreeding, since only the additive component of the genetic variance could be utilized. Since most of the response would be due to changes in the frequency of genes with additive effects, the hybrids between the F and S lines would be intermediate in development rate. Finally, the F line would approach still closer to the developmental limit, and as it did so the genetic component of the variance would decrease, although there would still be plenty of variation in the 'genetic score'.

There remains only the fact that the half-sib analysis of the foundation population failed to reveal any additive genetic variance. There were probably two reasons for this. First, analyses of this kind are necessarily inaccurate because of the relatively few degrees of freedom available. Second, since the foundation population was close to the developmental limit, not all differences in the 'genetic score' could be recognized phenotypically.

To sum up, earlier work had shown that much of the genetic variance of development rate is due to genes or to chromosome regions with heterotic effects. The present experiment shows that, at least in the geographically hybrid population studied, there was also some additive genetic variance, which could be utilized in selection for slow development. But this variance could not be used in selecting for fast development. This can be explained by saying, either that there exists a 'developmental barrier' preventing development in less than a certain minimum time, or, what amounts to the same thing expressed in genetic terms, that gene substitutions which in one part of the range of phenotypes are additive as between loci, are epistatic over another part of the range; there is a law of diminishing returns as genes for rapid development are introduced into the genotype.

It seems likely that developmental barriers of this kind, and the associated epistatic genetic variance, will prove to be common, particularly in the study of physiologically important characters which have been exposed to directional selection for some time. It is important to emphasize that there is nothing in the

least mystical about the concept of a developmental barrier. A hypothetical example will make this clear. It is known that in *D. subobscura* egg-laying accelerates the rate of ageing of females (Maynard Smith, 1958): a female laying eggs rapidly may survive for 60 days, whereas a genetically similar female laying eggs slowly or not at all may survive for 100 days. Starting from a population with a mean life-span of 60 days, selection for increased female longevity might perhaps produce a response, at the expense of reducing the rate of egg-laying, until a mean female life-span of 100 days was reached. Further progress would probably require much more profound physiological changes; if so, selective progress would be halted by a 'developmental barrier'. There is no reason to regard such barriers as absolute; for example, *D. melanogaster* develop appreciably faster than *D. subobscura* at the same temperature. But their nature in any particular case will call for a physiological rather than a genetical investigation.

SUMMARY

Starting from a geographically hybrid foundation population of *Drosophila subobscura*, selection for fast and for slow development has been practised without inbreeding on a diet with an unusually high level of protein. Realized heritabilities in the fast and slow lines were $+0{\cdot}063 \pm 0{\cdot}029$ and $+0{\cdot}186 \pm 0{\cdot}031$ respectively. A half-sib analysis of the foundation population and full-sib analyses of the first two and the last two selected generations were carried out. Hybrids between the two lines were approximately intermediate between their parents.

Two types of genetic explanation of the asymmetrical response are discussed. The first assumes directional dominance of alleles for fast development. Such an assumption can explain the asymmetrical response, but runs into difficulties in explaining the nature of the genetic variance in the selected populations and the intermediacy of the hybrids between the two lines.

A second assumption, which appears to fit the facts better, is that there exists a 'developmental barrier' preventing development at a rate appreciably faster than that of the foundation population. In physiological terms this implies that more rapid development requires a more profound modification of the population than could be achieved by a few generations of selection. In genetic terms, it implies epistatic interactions between genes at different loci: gene substitutions at a given locus which increase development rate on a genetic background causing slow development have little or no effect on a genetic background causing rapid development. In other words, there is a law of diminishing returns as more and more alleles for fast development are accumulated in the genotype. It is suggested that genetic situations of this kind may be common in populations which have been exposed to directional selection for a long time in reasonably large populations, either in nature or in domestication.

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Genic basis of the mate-killer trait in *Paramecium aurelia*, stock 540

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(*Received* 10 *August* 1960)

1. INTRODUCTION

Stock 540 of *Paramecium aurelia* (syngen or variety 1) is a 'mate-killer', i.e. individuals of this stock, when caused to conjugate with animals belonging to other ('sensitive') stocks, bring about the eventual death of all ex-conjugants deriving cytoplasm from the sensitive stocks (Beale, 1957). Such mate-killer paramecia contain in their cytoplasm large numbers of bacterium-like mu particles, and a clear association between presence of these particles and possession of the mate-killing trait was demonstrated. Details concerning the structure of the mu particles have been described by Beale & Jurand (1960), and preliminary genetic study showed that maintenance of the mu particles in stock 540 was dependent on the presence of a dominant gene *M*, which was absent in at least one sensitive stock (stock 60), lacking mu particles (Beale, 1957). Thus in essentials the mate-killing phenomenon of stock 540 conformed with what had previously been described of mate-killers in other stocks of *P. aurelia* belonging to syngen (variety) 8 (Siegel, 1953, 1954; Levine, 1953).

The object of the present investigation was to study in more detail the gene or genes necessary for maintenance of the mu particles in stock 540, and hence for the mate-killing properties of the host paramecia.

2. MATERIALS AND METHODS

The stocks used, all belonging to syngen 1 of *P. aurelia*, are listed below:

Stock No.	*Killer or sensitive*	*Origin*
540	Mate-killer	Mexico
168	Sensitive	Japan
119	,,	Pennsylvania, U.S.A.
217	,,	Florida, U.S.A.
513	,,	France
523	,,	Switzerland
544	,,	Louisiana, U.S.A.

All except stocks 513 and 523 were kindly supplied by Dr T. M. Sonneborn.

Scoring for presence of mu particles was done by crushing the paramecia between a cover-slip and a glass slide and examining the exudate under the phase-contrast

microscope, as previously described (Preer, Siegel & Stark, 1953; Beale, 1957). It has been repeatedly confirmed that presence of mu particles is a reliable indicator of the mate-killing trait of the host paramecium. In animals which had changed genotype at conjugation or autogamy, an interval of fifteen fissions was allowed to elapse before scoring for particles since Chao (1953) had found that the disappearance of kappa particles in stock 51 (syngen 4) following substitution of the allele *K* by *k* sometimes required this number of fissions, and similar evidence has been obtained by us for the mu particles of stock 540 (see below).

RESULTS

1. *Crosses between stock 540 (mate-killer) and six sensitive stocks of syngen 1*

With the aim of determining whether a number of sensitive stocks lacked the gene or genes necessary for maintenance of the mu particles, animals belonging to six stocks (all sensitive) of widely separated origins were crossed with stock 540.

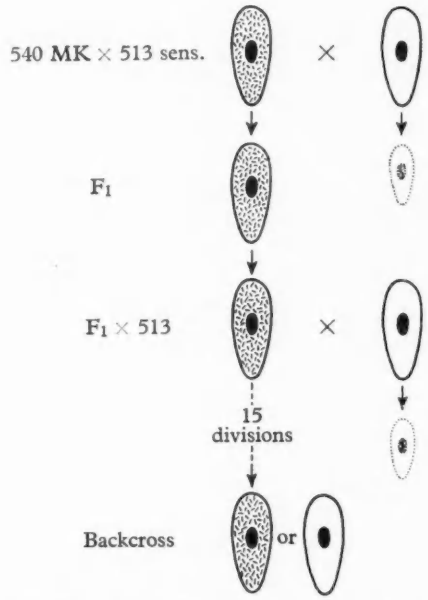

Fig. 1. Scheme of crossing stock 540 (mate-killer) and stock 513 (sensitive) to obtain segregation of *M* genes in backcross generation.

The surviving F_1 ex-conjugants, which were themselves all mate-killers, were then backcrossed to their respective sensitive parents, and the progeny classified in regard to possession of mu particles. The scheme of crossing is indicated in Fig. 1, and the results are summarized in Table 1. In addition to the backcross generations, some ex-autogamous F_2s were also raised, and are included in Table 1. They were

Table 1. *Progeny of hybrids between stock 540 (mate-killer) and six sensitive stocks*

Stocks crossed	Backcrosses of hybrids to sensitive stocks			Ex-autogamous F_2s		
	Mate-killers	Sensitives	Dead*	Mate-killers	Sensitives	Dead*
540 × 217	47	11	4	—	—	—
540 × 523	23	12	10	—	—	—
540 × 119	37	19	8	—	—	—
540 × 168	35	16	3	15	9	75
540 × 513	37	13	20	52	19	197
540 × 544 (*a*)	34	34	15	22	14	136
(*b*)	65	37	11	19	23	142
(*c*)	100	50	20	—	—	—
(*d*)	70	30	10	45	14	152

* These refer to clones which died due to some lethal genetic constitution and have nothing to do with mate-killing.

less useful than the backcrosses on account of the large amount (75–90%) of post-autogamous inviability found with these inter-stock hybrids.

The results showed that a proportion of sensitive animals was obtained in the backcross and F_2 generations from all combinations of stocks tested. It is therefore assumed that none of the six sensitive stocks contained all the genes necessary for maintenance of mu particles.

Few of the backcross data given in Table 1 suggest a 1 : 1 ratio of mate-killers to sensitives, such as would occur if stock 540 contained a single dominant gene *M* which was lacking in the sensitive stocks, as previously proposed (Beale, 1957). Crosses involving five out of the six sensitive stocks gave proportions not significantly differing from 3 mate-killers to 1 sensitive. (The sixth, stock 544, is considered below.) Such a 3:1 ratio would be expected if stock 540 contained two unlinked duplicate genes M_1 and M_2, either one alone being capable of maintaining the mu particles. In the backcross generation there would then be four genotypes ($M_1m_1M_2m_2$, $M_1m_1m_2m_2$, $m_1m_1M_2m_2$, $m_1m_1m_2m_2$) in equal proportions, only the last producing the sensitive phenotype. To test this hypothesis a more detailed study was made of the progeny from one series of crosses, that involving stocks 540 and 513.

2. *Further study of progeny from crosses between stocks 540 (mate-killer) and 513 (sensitive)*

An ex-autogamous F_2 family consisting of fifty-two mate-killer clones and nineteen sensitives was obtained from the cross 540 × 513 (see Table 1). According to the duplicate-factor hypothesis the fifty-two mate-killer clones should fall into three genotype classes ($M_1M_1M_2M_2$, $M_1M_1m_2m_2$, $m_1m_1M_2M_2$). To test this, ten of these F_2 clones (designated 'Testers 1–10') were taken at random, and each crossed and backcrossed to stock 513. The presence or absence of mu particles in the further backcross families thus obtained was noted and the results are given in Table 2.

Table 2. *Progeny of hybrids between stock 513 (sensitive) and ten 'tester F_2s' (mate-killers) derived from crosses between stocks 540 and 513*

Cross	Backcrosses of hybrids (tester F_2s × 513) to stock 513			Presumed theoretical ratio of mate-killers: sensitives
	Mate-killers	Sensitives	Dead†	
513 × Tester 1	72	28	4	3 : 1
2	79	21	5	3 : 1
3	58	42	7	1 : 1
4	62	38	10	3 : 1*
5	48	52	2	1 : 1
6	79	21	3	3 : 1
7	54	46	1	1 : 1
8	77	23	4	3 : 1
9	52	48	6	1 : 1
10	80	20	5	3 : 1

* See Table 3 for confirmation of genotype of Tester 4.
† See note in Table 1.

Five of the testers (Nos. 1, 2, 6, 8, 10) then gave backcross families in which the ratio of mate-killers to sensitives did not differ significantly from a 3:1, while four others (Nos. 3, 5, 7, 9) gave ratios not significantly different from a 1:1 ratio. (One tester, No. 4, gave indecisive results at this stage.) The testers 3, 5, 7, 9 were therefore presumed to contain only a single one of the dominant factors (M_1 or M_2), and testers 1, 2, 6, 8, 10 to contain both (M_1 and M_2).

Further crosses were then made between the four 'single factor' testers 3, 5, 7, 9, as shown in Table 3. The results indicated that testers 3 and 5 formed one group (denoted $M_1M_1m_2m_2$) and testers 7 and 9 a second group ($m_1m_1M_2M_2$). Inter-

Table 3. *Progeny obtained by intercrossing the 'tester' clones from an F_2 derived from stocks 540 × 513. After intercrossing the 'testers' the hybrids were passed through autogamy to yield a further ex-autogamous F_2. Some 'tester' hybrids were also backcrossed to stock 513*

Testers crossed	Nos. of mate-killer and sensitive progeny					
	Hybrids backcrossed to 513			Hybrids passed through autogamy giving F_2s		
	Mate-killers	Sensitives	Dead*	Mate-killers	Sensitives	Dead*
3 × 5				100	0	6
3 × 7				86	16	21
3 × 9				94	6	30
5 × 7	65	35	4	80	20	20
5 × 9	74	29	10	78	23	10
7 × 9				100	0	5
4 × 5	95	0	20			
4 × 9	86	0	20			

* See note in Table 1.

crossing of clones belonging to the different groups gave rise, after autogamy, to both mate-killers and sensitives.

Tester 4, whose genotype had been undecided from the results given in Table 2, was then crossed to each of testers 5 and 9, now known to belong to different groups. When these hybrids were backcrossed to a sensitive clone, only mate-killer progeny were obtained, indicating that tester 4 contained both dominant genes M_1 and M_2.

Since all the results from crosses between stocks 540 and 513 conformed with expectations based on the two-factor hypothesis (apart from a few minor numerical deviations), the hypothesis was considered proved.

3. *Further study of progeny from crosses between stocks 540 and 544*

Reference to Table 1 will show that while five of the sensitive stocks (217, 523, 119, 168, 513), after crossing to the mate-killer stock 540, gave results in good numerical agreement with the duplicate-factor hypothesis, stock 544 did not: there was a significant excess of sensitive clones over the one-quarter expected in backcrosses of 540/544 × 544. Further study was therefore given to these progeny. Ten F_2 clones (mate-killers) obtained by passage through autogamy of the hybrid 540/544 were designated 'testers 11–20' inclusive. The latter were each crossed to stock 544 and the hybrids backcrossed once more to stock 544 (Table 4). Four of these testers

Table 4. *Progeny obtained from 'tester' clones derived from an ex-autogamous F_2 from stocks 540 × 544. Each tester was crossed to stock 544 and the hybrid backcrossed to stock 544*

Stocks crossed	Nos. of progeny in backcross of hybrid to stock 544			Presumed theoretical ratio of killers : sensitives
	Mate-killers	Sensitives	Dead*	
Tester 11 × stock 544	64	35	4	2 : 1
12 ,,	70	28	6	2 : 1
13 ,,	48	49	3	1 : 1
14 ,,	65	34	0	2 : 1
15 ,,	69	31	1	2 : 1
16 ,,	60	35	2	2 : 1
17 ,,	52	48	8	1 : 1
18 ,,	51	46	9	1 : 1
19 ,,	44	43	14	1 : 1
20 ,,	76	35	3	2 : 1

* See note in Table 1.

(Nos. 13, 17, 18, 19) yielded 1 : 1 ratios of mate-killers to sensitives; the remaining six (Nos. 11, 12, 14, 15, 16, 20) (like the original F_2 from stocks 540 × 544) gave an excess of sensitives on an expectation of 3 mate-killers : 1 sensitive. These 'aberrant' figures suggested a 2:1 ratio. It was therefore postulated that one or other of the recombination classes, either $M_1M_1m_2m_2$ or $m_1m_1M_2M_2$, was lacking in these progeny. This supposition was checked by crossing each of the testers 11–20 with the two testers 5 and 9 derived earlier from crosses between stocks 540 and 513.

Tester 5 had previously been assigned the genotype $M_1M_1m_2m_2$ and tester 9 $m_1m_1M_2M_2$. Table 5 gives these results, which show that it is the group $m_1m_1M_2M_2$ which is missing. Six of the testers 11–20 belonged to group $M_1M_1M_2M_2$ and four to $M_1M_1m_2m_2$. While the numbers are not great, these results, taken together with the previous ones involving stocks 544 and 540, are consistent with the view that stock 544, though it evidently has the genotype $m_1m_1m_2m_2$, on crossing with stock 540 ($M_1M_1M_2M_2$) yields only three of the four expected classes, thus giving a 2:1 ratio of mate-killers to sensitives in ex-autogamous F_2s and backcrosses to the sensitive parent.

Table 5. *Progeny obtained by crossing testers 11–20 (from stocks 540 × 544) with testers 5 ($M_1M_1m_2m_2$) and 9 ($m_1m_1M_2M_2$)*

Cross	Backcross of hybrid to stock 544			Cross	Backcross of hybrid to stock 544			Inferred genotype of testers 11–20
Tester 5 ×	Mate-killers	Sensitives	Dead*	Tester 9 ×	Mate-killers	Sensitives	Dead*	
tester 11	62	0	33	tester 11	90	0	10	$M_1M_1M_2M_2$
12	75	0	25	12	76	0	24	$M_1M_1M_2M_2$
13	90	0	10	13	55	13	20	$M_1M_1m_2m_2$
14	84	0	16	14	58	0	22	$M_1M_1M_2M_2$
15	80	0	20	15	80	0	20	$M_1M_1M_2M_2$
16	90	0	10	16	81	0	19	$M_1M_1M_2M_2$
17	82	0	18	17	66	22	20	$M_1M_1m_2m_2$
18	58	0	42	18	46	6	50	$M_1M_1m_2m_2$
19	65	0	35	19	74	28	55	$M_1M_1m_2m_2$
20	81	0	19	20	76	0	22	$M_1M_1M_2M_2$

* See note in Table 1.

4. *Effect of mate-killer genotype on speed of killing*

As described above, any paramecium containing mu particles has been found to act as a mate-killer. However, it is possible that the number of particles present is to some extent related to the number of dominant *M* genes present. It has not been possible to count the particles in individual paramecia, but it is relatively easy to determine the time between conjugation of mate-killers with sensitive animals and death of the sensitive ex-conjugants. Levine (1953) had evidence that where the number of mu particles was large death of a sensitive animal was more rapid than where the number was small.

In Table 6, six different genotypes of mate-killers are compared in regard to the speed at which they killed sensitive paramecia following conjugation. Each combination was tested in two ways: (1) the mate-killer conjugant being mating type I and the sensitive being mating type II, and (2) the reverse.

After conjugation the sensitive ex-conjugant became gradually smaller and eventually vanished completely. The first observation when no animal could be seen was recorded as the 'time of death'. For times above 24 hours the results were not accurate to more than 12 hours.

Table 6. *Number of hours between conjugation and death of sensitive ex-conjugant (stock 544) following conjugation with mate-killers of various genotypes*

Mate-killer conjugant		Mate-killer conjugant mating type I Sensitive conjugant mating type II							Mate-killer conjugant mating type II Sensitive conjugant mating type I							Mean
Description	Genotype															
Stock 540	$M_1M_1M_2M_2$	4	6	10	8	11	10	10	10	11	10	8	10	10	12	12	10	9·5
Tester 9	$m_1m_1M_2M_2$	22	20	24	48	20	24	32	26	28	24	24	30	42	48			29
Tester 5	$M_1M_1m_2m_2$	20	24	32	22	22	24	30	22	36	40	48	26	22	24	34	30	29
F_1 540 × 544	$M_1m_1M_2m_2$	15	18	22	24	20	20	20	20	18	22	24	22	18	15	24	48	32	23
F_1 tester 5 × 544	$M_1m_1m_2m_2$	48	36	44	46	32	48	36	48		24	36	38	42	50				41
F_1 tester 9 × 544	$m_1m_1M_2m_2$	51	48	51	51	51	48	50	50		50	48	36	36	20	24			44

Notes: 1. Each figure in the Table refers to time for one ex-conjugant animal.
2. Most times above 24 hours accurate to nearest 12 hours only.

In these experiments occasional pairs were obtained apparently yielding two viable ex-conjugants, but by studying the behaviour of marker genes (controlling the antigens) it could be shown that in these cases no exchange of nuclear material had taken place. Wherever there was true conjugation, not merely an abortive contact between a mate-killer and a sensitive paramecium, unilateral death followed.

The results given in Table 6 show clearly a proportionality between number of *M* genes and speed of killing, from 9·5 hours with four *M* genes to about 44 hours with one *M* gene. There was no difference in killing effect of particular mating-types, of given genotype.

6. *Number of fissions between loss of* M *genes and disappearance of mu particles*

When heterozygous mate-killers (of genotype $M_1m_1M_2m_2$ or $M_1m_1m_2m_2$) were passed through autogamy or backcrossed to sensitive animals of genotype $m_1m_1m_2m_2$, the surviving animals having the genotype $m_1m_1m_2m_2$ (and cytoplasm derived from the mate-killer parent) were found not to lose the mu particles immediately, but only after a delay lasting an appreciable number of fissions. However, when the loss of particles did occur it seemed to take place abruptly: cells either had a large number (>100) of particles or none at all.

A detailed study of the rate of appearance of animals lacking particles due to change in genotype will be the subject of a future publication. All that we wish to state here is that, from preliminary tests, the mu-less animals appeared at times between the eighth and fifteenth post-conjugational fissions for crosses of the type $m_1m_1M_2m_2 \times m_1m_1m_2m_2$, the mean being about twelve fissions, and following crosses of the type $M_1m_1M_2m_2 \times m_1m_1m_2m_2$, the mu-less animals appeared between eleven and fifteen fissions after conjugation, with a mean of 13·5 fissions.

DISCUSSION

The relationship between the mu particles and the two dominant duplicate genes M_1 and M_2 has no exact parallel in other types of killer paramecia. In syngen 4, Sonneborn (1947) and Balbinder (1956) have shown that certain stocks contain, in addition to the gene *K*, genes denoted S_1 and S_2 which modify the capacity of the animals to maintain kappa particles. Paramecia containing *K* and in addition S_1 or S_2 are more likely to lose the particles than animals containing the alleles s_1 and s_2; but here *K* is the only gene known to be essential for maintenance of kappa. The existence in stock 540 of two genes, M_1 and M_2, either one of which is capable of supporting the growth of mu particles, is difficult to understand from an evolutionary standpoint, especially since so far no other stocks of syngen 1 have been found to contain either of these genes. One interpretation is that these genes have some function unconnected with maintenance of mu particles, and it is on the basis of this other function that natural selection has operated to secure the establishment of the genes in stock 540. Then at some later period infection by mu particles took place, and it became apparent that these genes produced a satisfactory 'cytoplasmic environment' for the particles.

Examples of duplicate factors are rather rare in the genetical literature (apart from polyploids), and it may be, as Sonneborn (personal communication) has pointed out, that stock 540 has one pair of chromosomes duplicated. The diploid chromosome number of *P. aurelia* is known to vary considerably between different stocks of a single syngen (Dippell, 1954). However, this interpretation becomes more difficult to accept when one considers that there is independent assortment of the two pairs of alleles M_1–m_1 and M_2–m_2, indicating a lack of homology of the chromosomes concerned.

The anomalous behaviour of stock 544, following crosses with stock 540, may be interpreted as follows. It is assumed that the chromosomes of stock 544 are involved in a translocation, whereby the two recessive alleles m_1 and m_2 are linked together. On crossing with stock 540, which bears the alleles M_1 and M_2 on separate chromosomes, and subsequently backcrossing to 544, individuals will be expected to arise containing duplications or deficiencies. Depending upon the relative lengths of the segments containing m_1 and m_2, positions of the centromeres, etc., one might expect the occurrence of unbalanced types $M_1m_1m_2$ (deficiency) and $M_2m_1m_2m_1m_2$ (duplication). It is possible that the former would be inviable and the latter viable. In view of the unsuitability of *Paramecium* for cytogenetic work, it does not seem profitable to pursue this matter further.

Turning now to the relationship between genes and particles, and between genes and their phenotypic effect, we find somewhat similar results to those of Chao (1953), working with killer paramecia in syngen 4. Chao found that the homozygotes KK contained approximately double the number of kappa particles found in the heterozygotes Kk. Although we were not able to count the mu particles in paramecia of different genotypes, a proportionality between number of M genes and speed of killing was found, and the latter is probably related to the number of particles. These variations in particle number have to be considered against a standard environment, since it is known that factors such as temperature and growth rate markedly affect the number of particles. In stock 540, starvation, for example, results in a dramatic increase in the concentration of mu particles.

The disappearance of mu particles which takes place when an animal of genotype $m_1m_1m_2m_2$ is derived from $M_1m_1m_2m_2$ at conjugation or autogamy, apparently occurs only after a delay of 8–15 fissions, which agrees remarkably well with the range found by Chao (1953) for the disappearance of kappa particles in stock 51, syngen 4. Moreover, in both cases loss of particles, when it does occur, is abrupt. There is not, as might be expected, a simple dilution of the particles from cell generation to generation, whereby the number per animal is halved at each fission.

These facts bear on the manner in which the genes M_1 and M_2 support growth of the mu particles, and a fuller discussion of this important problem will be given later.

SUMMARY

1. Stock 540 of *Paramecium aurelia* (syngen or variety 1) contains two duplicate genes M_1 and M_2, each of which is capable of supporting growth of mu particles in the cytoplasm, thus producing the mate-killer phenotype.

2. Of six sensitive stocks of *P. aurelia* syngen 1, collected from widely separated localities, none contained either M_1 or M_2.

3. One sensitive stock (544), though proved to contain both recessive genes m_1 and m_2, gave aberrant ratios of mate-killers and sensitives following hybridization with stock 540. Evidence has been adduced consistent with the hypothesis that the m_1- and m_2-bearing chromosomes are involved in a translocation, by comparison with stock 540.

4. There is a proportionality between numbers of M genes and speed of killing, and presumably therefore with the number of mu particles.

5. Loss of mu particles following substitution of M genes by their recessive alleles occurs at times varying between eight and fifteen fissions after change of genotype.

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Linkage relations and some pleiotropic effects of the dreher mutant of the house mouse

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The mutant *dreher*, *dr*, of the mouse *Mus musculus* L. was first reported by Falconer & Sierts-Roth (1951). They described the circling behaviour of the affected animals and showed that the character was due to a recessive gene, not allelic with *v*, *sh*-1, *sh*-2, *je*, *kr*, *pi* or *fi* and not closely linked with W^v. Subsequently, Fischer (1956) described the morphological defects of the inner ear which are the basis of the dancing behaviour, and analysed their embryology (Fischer, 1958), while Bierwolf (1958) studied the accompanying defects of the brain.

The present paper describes the linkage relations of dreher and some previously unknown pleiotropic effects.

LINKAGE TESTS

Dreher homozygotes, from a stock kindly supplied by Dr D. S. Falconer, were crossed with animals from various linkage-testing stocks. To test linkage with recessive markers the F_1 animals were intercrossed, and in the case of dominant markers they were backcrossed to homozygous *drdr*. The results are shown in Tables 1 and 2. The original tests indicated possible loose linkages with *Re*, *T* and

Table 1. *Linkage tests of dreher with dominant markers* (M)

Marker	Heterozygote type	Offspring					χ^2	Recombination (%)
		M+	++	*Mdr*	+*dr*	Total		
Ca	C	52	45	46	55	198	1·29	46·0 ± 3·5
Mi^{wh}	C	54	44	51	49	198	0·32	48·0 ± 3·6
Ra	C	30	29	43	31	133	0·91	54·1 ± 4·3
Re	C	60	42	46	53	201	3·11	43·8 ± 3·5
	R	34	32	26	17	109	0·45	46·8 ± 4·8
Sd	C	21	40	7	28	96	0·04	49·0 ± 5·1
T	C	29	57	47	39	172	7·53	60·5 ± 3·7
	R	43	59	52	46	200	2·42	44·5 ± 3·5
W^v	C	39	56	46	47	188	1·36	54·3 ± 3·6

C = coupling; R = repulsion.

ln. The linkages with *Re* and *T* were not confirmed when the tests were repeated using repulsion heterozygotes. The linkage with leaden (*ln*) in linkage group XIII,

Table 2. *Linkage tests of dreher with recessive markers* (m)

Marker	Offspring ++	+m	dr+	drm	Total	χ^2	Recombination (%)
a	19	3	8	—	30	0·73	38·3 ± 13·7
b	95	31	19	13	158	2·70	59·8 ± 6·0
c^{ch}	86	40	19	13	158	0·48	54·1 ± 6·0
d	103	23	22	10	158	2·36	59·2 ± 6·0
fz	203	55	55	10	323	0·47	47·1 ± 4·2
ln	185	73	57	8	323	6·08	39·7 ± 4·2
p	66	24	16	5	111	0·08	48·0 ± 7·1
s	101	25	27	5	158	0·07	48·4 ± 6·0
vt	68	22	15	6	111	0·12	52·5 ± 7·1
wa − 2	68	22	17	4	111	0·17	47·1 ± 7·1

All heterozygotes were of the repulsion type. Recombination values and χ^2's were calculated by the method of Fisher (1946).

however, was confirmed by a test with dominant hemimelia, *Dh*, which Carter (1957) reported to be linked to *ln* with a recombination value of about 6%, the order of genes being *Dh–ln–fz*.

Threepoint backcrosses were made using heterozygotes of the type *dr* + +/+ *Dh ln* (Table 3). In these crosses dreher showed 27·2 ± 3·0% recombination with *Dh* and 27·7 ± 3·0% with *ln*. Two possible orders of the three genes which

Table 3. *Data obtained from threepoint backcrosses of the type* dr + +/+ Dh ln × dr + ln/dr + ln. *The data from female and male heterozygotes are shown separately*

Phenotype of progeny	No. of young from ♀ heterozygote	♂ heterozygote	
dr + +	70	5	Non-crossovers
+ *Dh ln*	71	7	153
+ + +	32	0	*dr* crossovers
dr Dh ln	20	3	55
dr + *ln*	3	0	*ln* crossovers
+ *Dh* +	2	0	5
dr Dh +	0	0	Double crossovers
+ + *ln*	4	0	4

would be compatible with these figures are *Dh–ln–dr* and *dr–Dh–ln*, the latter being the more probable. The first of these is ruled out since it would entail close linkage of *dr* with *fz*, which was not found. If the second order is accepted, then animals of the phenotype + + *ln* represent double crossovers, and it is surprising that four of these should have been found when the two intervals, *dr–Dh* and *Dh–ln*, are quite short. However, Searle (1959) found that occasional *Dh* heterozygotes have

completely normal limbs, and since in this experiment *Dh* was classified by limb abnormality only, it is possible that these four animals were *Dh*+ normal overlaps. If so, their genotype was $+Dh\ ln/dr+ln$ and they were non-crossovers. No double crossovers of the complementary type were found; there is thus no bar to accepting the second order, *dr–Dh–ln*, and it will be considered to be established. This means that *dr* is close to *Lp* and to the translocation break in T190, but its position in relation to these is not known. Normal overlapping by *Dh* will tend to increase the apparent recombination between it and *dr*, and a more accurate value for the recombination fraction will be obtained by considering only the manifest *Dh* young. In the backcross experiment there were 103 *Dh* young, of which 23 were also dreher and therefore crossovers. This gives a recombination value of $22{\cdot}3 \pm 4{\cdot}1\%$. Among the same *Dh* animals the *Dh–ln* recombination was $1{\cdot}9 \pm 1{\cdot}3\%$ and the *dr–ln* value was $24{\cdot}3 \pm 4{\cdot}2\%$.

PLEIOTROPIC EFFECTS

During the course of the linkage tests two previously undescribed pleiotropic effects of the *dr* gene became apparent.

The first of these effects to be noticed was a tendency to white spotting; many homozygous dreher animals have a partial belt of white. In families in which dreher was segregating but no known spotting genes were present, 54 out of 66 dreher young had some white spotting in the belt region whereas only 3 out of 103 non-dreher animals showed any. The effect in the drehers was confined to the belt region but was variable in extent. Of the 54 spotted drehers, 6 had a complete belt, 24 had ventral belts only, 16 had ventral belts which extended up the flanks, and 8 had merely belly spots. The three non-dreher animals with spotting had belly spots only; to find 3 out of 103 otherwise fully pigmented animals with belly spots is within the range of normal variation.

The second pleiotropic effect of the dreher gene to be noticed was a tendency to modify the expression of the gene brachyury (*T*). Animals of the genotype $T+drdr$ had on the average shorter tails than those of the genotype $T++dr$ born in the same family. In the progeny of some matings segregating for *T* and *dr* the tail length of the brachyury animals was estimated by eye, as a fraction of the normal length. The non-dreher brachyurics in this stock typically had tails which were about nine-tenths of normal length, but the dreher brachyurics had in general tails of less than half normal. Table 4 shows that the mean of the estimated lengths of the non-dreher tails was 0·88 normal whereas that of the drehers was 0·38 normal.

Table 4. *Estimated lengths of tails of* T+ *animals heterozygous and homozygous for* dr, *expressed as tenths of normal length*

	No. of mice with tails of x tenths length											
Genotype	$x=$ 0	1	2	3	4	5	6	7	8	9	Total	Mean
$+dr$	—	—	—	—	—	1	1	3	7	47	59	0·88
$drdr$	11	5	7	9	8	8	3	2	5	6	64	0·38

Such an interaction of *dr* and *T* was quite unexpected since *dr* alone had no externally visible effect on the tail or skeleton in general. Further, the interaction still remained unexplained when alizarin-stained preparations of the skeletons of three animals of each of the four types $T+\ +dr$, $T+\ drdr$, $+\ +\ +dr$ and $+\ +\ drdr$ were made. No skeletal abnormalities which could account for the effect were found.

In both these types of pleiotropism there were some drehers which did not show the effect. This could have two explanations. Either the pleiotropic effects in question show incomplete penetrance or they are not due to pleiotropy but to a gene linked to *dr*, the drehers not showing the character being crossovers. The second explanation is ruled out since the hypothetical complementary crossover types (non-dreher belted animals, or non-dreher $T+$ animals with tails less than half normal length) were not found. Therefore the failure of some drehers to show the effects must be due to incomplete penetrance. It still remains possible that this was not a case of true pleiotropy but that genes very closely linked to *dr* were responsible. It seems best to accept the hypothesis of pleiotropy until it is disproved by the finding of true crossovers.

SUMMARY

Linkage studies with the mutant dreher (*dr*) of the mouse (*Mus musculus* L.) have shown that it lies in linkage group XIII and that the order of loci is *dr–Dh–ln*. The recombination between *dr* and *Dh* was $22{\cdot}3 \pm 4{\cdot}1\%$.

Two previously undescribed pleiotropic effects of the *dr* gene were observed. The first was a tendency to white spotting in the belt region, and the second an interaction with brachyury (*T*) such that *drdr T+* animals had shorter tails on the average than *+dr T+*.

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Polygene analysis

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This paper considers why polygene practice is less successful than polygene theory. It introduces an alternative approach to the analysis of quantitative inheritance.

DISCUSSION

It is usual to argue from discrete Mendelian genes to discrete polygenes. This is not strictly valid since Mendelian genes form an extremely unrepresentative sample of the total genetic material. They are mutants of quite exceptional effect which do not happen to be lethal and which show good penetrance. In other words, we select them from the total of all mutants precisely because they have large, independent effects. We might nevertheless suppose that the chromosome is one long graded functional unit which cannot meaningfully be chopped into a series of discrete non-overlapping functional units. But microbial genetics provide evidence against this supposition. Pritchard (1955) finds a map-length between functional units (genes) of the same order as that between mutational sites within genes. Genes are therefore discrete. But the same evidence shows that recombination within the gene is at least as frequent as recombination between genes. This may not matter if the genes are Mendelian, for the products of recombination within a Mendelian locus can be expected to give roughly the same effects as one or other of the original alleles, rather than some intermediate effect. This is not true of genes controlling smaller differences; given two alleles determining two forms (of differing efficiencies) of the same enzyme, within-gene recombination can and does lead to the formation of enzymes of intermediate efficiencies. Thus, if polygenes are identified with functional units, within-gene recombination (which happens relatively often) can give quite different alleles from the original pair. If, on the other hand, polygenes are taken to be the mutational sites, they certainly do not act additively even at enzyme level, let alone at the level of the complete organism; their interactions (statistically speaking) are as large as their main effects.

This impasse can be avoided by forgetting about genes and starting at the other end of the scale. Statistical analysis of progeny means of crosses between various parents invariably shows that complete genomes have approximately additive effects; interactions, although they occur, are less important than main effects. The same is true of the effects of individual (marked) chromosomes. We may therefore hope that it will also be true of pieces of chromosomes. It must be expected that (since a genotype is a closely integrated structure) the smaller the pieces, the more important interactions will be. The trouble is, of course, that (unlike other

applications of the statistical method of fitting constants) we do not know which individuals contain which pieces of chromosome.

Mangelsdorf (1952) expects that in any individual, development of a particular character will be limited by a small subset of all the genes concerned with that character, and that different individuals will be limited by different subsets. Mendelian genes are characterized by the peculiarity that the subset is the one gene for all individuals. Thus Harborne (1960) finds that a gene controlling a particular flower pigment does so by causing the hydroxylation of a flavonol. There must obviously be a lot of indispensable genes concerned with the production of the flavonol in the first place, yet we are pleased to call this hydroxylating gene 'the' gene for this particular flower colour! Mangelsdorf's quite reasonable idea emphasizes the necessity of the pragmatic approach by 'lengths of chromosome'. If polygene analysis on this basis were found to work, it would be just as rash to argue from it to conclusions about gene action as to argue from the successful use of main effects in fertilizer trials to conclusions about nitrogen metabolism inside the plant. Estimates of polygene 'linkage' or 'unfixable genetic variation' should, I submit, be taken with a plentiful dose of salt. Fortunately, except perhaps in the case of heterosis, the breeder is exclusively concerned with phenotypes. Polygene analysis, regarded in this way as an attempt to analyse chromosomal effects, still offers some hope as a method of prediction. The question of homozygosity may also be considered in this light. Although it is unlikely that the mathematical theory of inbreeding is exactly fulfilled in practice, the remarkable effects of inbreeding on normally outbreeding organisms leads us to expect that the difference between two homologous chromosomes of an inbred line will be much less than differences between two chromosomes taken from different strains, and this is all that is required to justify the working assumption that inbred parents are homozygous.

In the simplest case of hypothetical strains of *Drosophila* with exactly one crossover per chromosome in the female (and none in the male) we would be dealing in the F_2 and first backcross generations with chromosomes, each derived as one length from one parent joined to one length from the other parent. These lengths will of course differ in different individuals. Chromosomes composed of four shorter lengths (of the parental chromosomes) would appear in the F_3. It would be better to use means, rather than variances, when comparing different generations; for the chromosome pieces are of different lengths in different individuals and in different generations, and this will affect the variances but not, so much, the means. It may also be noted that the number of 'effective factors' will be at least twice or thrice the haploid chromosome number. As long as estimates of this number of effective factors take impossibly low values, little confidence can be placed in the other results of polygene analysis. The more complicated an analysis, the more desirable it is to be able to check on the plausibility of the model employed. The number of effective factors is the only criterion available here, so that it is very dangerous to gloss over the unfortunate estimates actually obtained.

In polygene algebra it is usual to assume that any gene substitution has a particular effect on the phenotype irrespective of the composition of the rest of the genotype.

Any deviation from this is treated as gene interaction. However, it seems reasonable to suppose that the effect of a 'good' gene may be less in a 'good' genotype than in a 'bad' one; in other words, that a law of diminishing returns will operate. (The word 'gene' here means 'chromosome length' as above.) Rasmusson (1933) explores one aspect of this idea. Such curvature of the gene response-curve may certainly be described in terms of interaction between additive genes, but only as a combination of many small interactions of all orders, so that in practice the additive way of thinking is very poorly adapted to the situation. It appears that polygene analysis is peculiarly sensitive to this type of interaction. As an example, we may consider a case of eight fully dominant genes, each with effect +1, in an experiment involving F_2 and backcrosses. The genes all act in the same direction in each parent, so that if the value for the smaller parent is 100, that for the larger is 108. An 8% difference between parents seems reasonable in breeding material. The linear and non-linear gene response curves are taken to be:

	Number of genes								
	0	1	2	3	4	5	6	7	8
	Phenotype								
A	100	101	102	103	104	105	106	107	108
B	100	102·1	103·8	105·1	106·1	106·9	107·4	107·8	108
C	100	100·97	101·94	102·93	103·92	104·93	105·94	106·97	108

Here A is the additive case, B a case of 'diminishing returns', and C a purely multiplicative system where each successive gene multiplies the phenotypic value by 1·00967. Since we know that the genes all act in the same direction in each parent, we are entitled to estimate the number of effective factors. The means, variances, and estimates of D, H and k are:

	A		B		C	
	Mean	Variance	Mean	Variance	Mean	Variance
Parent 1	100	0	100	0	100	0
Parent 2	108	0	108	0	108	0
F_1	108	0	108	0	108	0
B_1	104	2	105·8	1·816	103·93	2·000
F_2	106	1.5	107·3	0·451	105·95	1·553
B_2	108	0	108	0	108	0
D	2		−1·83		2·21	
H	2		5·46		1·79	
k	8		−8·7		7·2	

Example A of course fulfils the assumptions of the analysis perfectly, and so the estimates are correct. Example B is quite severe, but not at all unreasonable. As may be expected, a local flattening or steepening of the gene response curve alters the variances considerably, but the means only slightly. The estimates of D

and H, obtained as differences between multiples of the variances, are affected even worse. In example C there is a very slight deviation from the additive model in any individual phenotype, amounting to at most 0·08% (or 1% of the difference between the two parents). The means are affected to a similar extent, but one of the variances is increased by 3·5% and the estimates of D, H and k have consequently altered by 10%. This hardly bears out the opinion of Mather (1949) that 'a small departure from additiveness is not in any case likely to engender serious difficulties or errors'. Small but systematic departures can cause big trouble. A similar conclusion applies to other estimates of the number of effective factors, for such estimates are usually the square of a statistic of order n divided by another statistic of order $2n$.

Now, as Mather (1952) points out, scaling tests are an essential part of the analysis. The means in examples B and C fail two of the three possible scaling tests and consequently it is not permissible to make the estimates of D, H and k until the data have been transformed. Unfortunately, the above examples show that, since the means are so much more stable than the variances, scaling tests may be satisfied to within the limits of experimental error while the variances remain quite misleading. Examples in the next section, using real data, show that genuine (and recognizable) departures from additivity can occur which the scaling tests are too insensitive to pick up. In practice the means must be estimated more accurately than they are at present if scaling tests are to be of any use. A further objection is that a particular scale may appear to suit a given set of data, but might have failed to satisfy scaling tests on further crosses (involving the same material) which have not in fact been grown. This objection, trivial until the extreme importance of scaling tests is realized, appears to be insuperable. There is of course no guarantee that a scale that suits the genes *on average* will suit each individual gene. This objection is minimized if we consider only the means, not the variances.

It is obviously important, in any type of statistical analysis, to use a method that not merely gives the right answer if the assumptions involved are exactly fulfilled, but is not ruined by reasonable deviations from these assumptions. Such methods are called 'robust' by Box & Andersen (1955), who discuss the robustness of various commonly-used statistics. We see that polygene analysis, which uses the means to provide a check and the variances as sources of estimates, is not robust since the variances are considerably more sensitive than the means. It is in fact well known to professional statisticians that the variance-ratio and Bartlett's test, concerned with variances *per se*, are more sensitive to deviations from Normality than is the t-test. Once again, it would be better to estimate from the means and use the variances as a check. As in normal statistical practice, transformations would then be used only if demanded by the error structure (or as an aid in solving maximum likelihood equations). Since the situation is sensitive to curvature of the gene response curve, it seems reasonable to allow for such curvature in the analysis. Of course, it cannot be expected that such an analysis will more than approximate to the true situation, but it may be sufficiently robust to permit useful estimation of possible short-term selection results. On the other hand, it may not. The matter is

explored further in the next section, but it is clear that little confidence can be placed in the detailed results of polygene analysis in its present form. 'If you get on the wrong track with the Mathematics for your guide, the only result is that you get to the Valley of Mare's Nests much quicker; get there so smoothly and easily that you do not realize where you are and it may be hard to unbeguile you' (Yule, 1920).

ALGEBRA

This section explores a way of increasing the robustness of polygene analysis. It appeared above that it would be preferable to use family means, rather than variances, for estimation. This is impossible as long as the additive hypothesis is adopted, since this hypothesis imposes certain constraints on the means. If the means actually observed do not satisfy these constraints ('scaling tests') the only thing to do is to transform the data as directed by these scaling tests. Unfortunately, these tests have to be very precisely satisfied before we can have much confidence in estimates derived from the variances. An alternative approach is to loosen the constrictions imposed by the additive hypothesis. I shall consider here the substitution of a gene response curve of the type $(a+x)\,\theta^x$ instead of the additive $(a+x)$. This new function, by combining additive and multiplicative gene action, can give a wide range of curves of different types, including (1) a 'diminishing returns' curve ($\theta < 1$) where the effect of any one gene decreases as the phenotype departs from the origin a, (2) the additive system ($\theta = 1$), and (3) a multiplicative type of action ($\theta > 1$, x/a small). It leads to tractable—even elegant—algebra and can, of course, be extended to include sines and cosines by taking imaginary values of θ. It may turn out that, by fitting such a flexible hypothesis to the observed means, a sufficiently good approximation to the true situation can be made. Extreme caution is necessary since, the more complicated a statistical analysis, the less trustworthy are its results (and conversely, the simpler an explanation of a biological phenomenon, the more superficial it is likely to be). All the same, it is worth while attempting direct estimation from the means. The advantages include the statistical robustness discussed in the previous section, the fact that we can average over the effects of chromosomes broken by different numbers of crossovers and at different places (whereas the variances must be inflated as a consequence of the variability of crossover positions) and the consideration (since we are interested in predicting genetic advance under selection) that prediction from means to means is likely to be safer than from variances to means when the assumptions on which the analysis is based go wrong.

I shall consider a simple experiment consisting of two (homozygous) parents, their F_1, F_2 and two backcrosses B_1 and B_2. Of the $(f+g)$ loci at which the parental genotypes differ, $f\,(>g)$ are homozygous for 'good' alleles in P_1. The phenotype of an individual homozygous for l 'good' alleles, n 'bad', and heterozygous at m loci is

$$(b+\overline{l-n}\,d+mh)\,\theta^{l-n}.$$

Then the generation means are:

P_1 $[b+(f-g)d]\theta^{f-g}$

F_1 $b+(f+g)h$

B_1 $\left[b+\dfrac{f\theta-g}{1+\theta}d+\dfrac{f+g\theta}{1+\theta}h\right]\theta^{-g}\left(\dfrac{1+\theta}{2}\right)^{f+g}$

F_2 $\left[b+\dfrac{\theta-1}{1+\theta}(f+g)d+(f+g)\dfrac{2h\theta}{(1+\theta)^2}\right]\theta^{-f-g}\left(\dfrac{1+\theta}{2}\right)^{2f+2g}$

P_2 and B_2 are obtained by exchanging f and g in the expressions for P_1 and B_1.

At first sight, we may hope to omit the dominance term; apparent dominance might be explained by curvature of the gene response curve. But we shall see below that published data require the retention of a term for dominance. The means may then be written (approximately) as:

P_1 $(b+2d_2)x^2$

P_2 $(b-2d_2)x^{-2}$

F_1 $b+2h_1$

B_1 $(b+d_1+d_2+h_1+h_2)x$

B_2 $(b+d_1-d_2+h_1-h_2)x^{-1}$

F_2 $b+2d_1+h_1$

F_3 $b+3d_1+\frac{1}{2}h_1$

where $2d_1=\dfrac{\theta-1}{\theta+1}(f+g)d$, $2d_2=(f-g)d$,

$2h_1=(f+g)h$, $2h_2=\dfrac{1-\theta}{1+\theta}(f-g)h$,

$x=\theta^{\frac{1}{2}(f-g)}$ and $\left(\dfrac{1+\theta}{2}\right)^2$ is approximated by θ.

These expressions are symmetric in the sense that P_1 and P_2 can be interchanged by reversing the signs d_2 and h_2 and reciprocating x. Evidently d_1, d_2, h_1, h_2 represent average genetic effects. At first sight it seems that if $\theta=1$, $x=1$ and $d_1=h_2=0$. Further consideration of these formulae shows, however, that x represents a general curvature of the scale of measurement while d_1 is a kind of interaction between $(\theta-1)$ and d; it is in fact concerned with the possibility that the size of the contribution $\pm d$ may be different for 'positively' and 'negatively' homozygous loci. Incidentally, it should be noted that d_1 becomes more important in later generations with greater scatter of the genotypes. Thus x, d_1 and h_2 each measure their own characteristic kind of departure from additivity; it is quite possible to have $x=1$ and yet find non-zero values for d_1, h_2. On the face of things we would expect h_2 (a second-order interaction) to be less important than x or d_1. If we regard x and d_1 as independent entities in their own right, we are freed of the assumption (made above implicitly) that all genes have the same numerical effect.

Now

$$F_2^2-B_1B_2=(d_2+h_2)^2+d_1(2b+3d_1+2h_1),$$

so that negative values of d_1 permit the F_2 mean to be significantly less than the geometric mean of the two backcrosses. It may be that these cases arise in practice only for artificial characters like average fruit weight where the natural direction of increase of the denominator (fruit number) is reversed. For clearly, if

$$F_2^2 > B_1 B_2, \quad \text{then} \quad \left(\frac{1}{F_2}\right)^2 < \frac{1}{B_1} \cdot \frac{1}{B_2}.$$

None the less, we still want to be able to analyse artificial characters as they stand, at any rate until it transpires that separate analyses of numerator and denominator are obligatory. Now, such cases are impossible in additive polygene analysis, for the scaling equalities

$$2B_1 = P_1 + F_1, \qquad 2B_2 = P_2 + F_1,$$
$$4F_2 = 2F_1 + P_1 + P_2$$

lead immediately to $16(F_2^2 - B_1 B_2) = 4(B_1 - B_2)^2 = (P_1 - P_2)^2$. There are four published cases (Mather, 1949; and Smith, 1952) which satisfy these scaling tests and also permit us to compare these three quantities.

	Mather, p. 44		Smith, p. 164	
	$D \times J$ 1938	$D \times J$ 1939	Plant height	Leaf length
$16(F_2^2 - B_1 B_2)$	26·90	52·47	−1022·4	−32·00
$4(B_1 - B_2)^2$	14·82	8·36	492·8	4·00
$(P_1 - P_2)^2$	13·32	8·09	364·8	1·21

It appears that these three estimates of the same genetic trait can be quite contradictory even when the scaling tests are satisfied to within sampling error. $(P_1 - P_2)^2$ is used in estimating the number of effective factors. Since from the genetical point of view we could equally well use either of the other expressions (or a combination of all three), it appears that the estimate is to a large extent at the choice of the experimenter. The trouble is, of course, that $F_2^2 - B_1 B_2$ is the relatively small difference between two large quantities, so that an insignificant change in F_2, B_1 or B_2 can convulse $F_2^2 - B_1 B_2$. As may be expected, $4(B_1 - B_2)^2$ comes out similar to $(P_1 - P_2)^2$, but sometimes so much bigger as to alter seriously the estimate of the number of effective factors.

The negative values of $F_2^2 - B_1 B_2$, although not significant, suggest that Smith's acceptance of an additive genetic system needs re-examination. Putting non-significant parameters zero, we find that for plant height $b = 37{\cdot}5$, $h_1 = 3{\cdot}1$, $x = 1{\cdot}140$, and for leaf length $b = 11{\cdot}15$, $x = 1{\cdot}032$. These figures give expectations:

	Plant height		Leaf length	
	Observed	Expected	Observed	Expected
P_1	47·8	48·8	11·6	11·87
P_2	28·7	28·8	10·5	10·47
F_1	43·2	43·7	11·1	11·15
F_2	40·6	40·6	11·2	11·15
B_1	47·3	46·3	11·8	11·51
B_2	36·2	35·6	10·8	10·80

In the case of plant height the additive dominance term could be replaced by a multiplicative type, so that it appears that a multiplicative genetic system would be more appropriate to these sets of means than an additive system. *The scaling tests are not good enough to guarantee additivity.* The $D \times J$ 1938 data used by Mather provide a further example. In the following analysis I have pooled the errors of the six means and have consequently used unweighted least squares. Fitting the additive model (i.e. setting $d_1 = h_2 = 0$, $x = 1$), the goodness-of-fit χ^2 is 5·207 (3 d.f.), which bears out the result of the individual scaling tests that no departure from additivity is apparent. However, closer investigation shows that a non-additive model fitting d_1 but keeping $h_2 = 0$ and $x = 1$ reduces this value of χ^2 to 0·760 (2 d.f.), i.e. the χ^2 due to fitting d_1 is 4·447 (1 d.f.). This procedure certainly involves an element of selection, but nevertheless it again appears that the new analysis is capable of picking up deviations from additivity that pass unnoticed by the scaling tests, i.e. is more sensitive than the additive analysis.

Now it can be seen that as long as we restrict ourselves to the first and second generations, it will be difficult to separate the effects d_1 and h_1 accurately. This means that any practicable polygene experiment restricted to F_2 and first back-cross will, however analysed, be rather insensitive to interactions of the type epitomized by d_1. Such interactions are precisely the sort best calculated to ruin predictions of genetic advance. To avoid this impasse, further generations must be included even though the parental chromosomes are further broken up by recombination and there is now the possibility of selection. Comparison of F_1, F_2 and F_3 means gives a direct test of the existence of d_1, independent of x.

It may be objected that, with the curved gene response, an arbitrary change of origin is no longer entirely absorbed by a corresponding change in b. This difficulty will not be very serious if $x \simeq 1$, and in any case there is usually a natural origin. Another point is that where F_2^2 significantly exceeds $B_1 B_2$, two solutions are possible. This difficulty, which again can be removed by including the F_3, is unimportant since the alternative solution is an improbable one wherein x is considerably different from 1.

CONCLUSION

There are two possible ways of meeting the difficulties. One is to retain the usual polygene analysis of second-degree statistics, but paying much more attention to the question of scale. The scale should be tailored to fit the observed means, rather than be taken ready-made from a book of mathematical tables. It is clear that once the curved model has been fitted to a set of means, a back transformation can be used to return to an additive model. If, from the infinity of possible transformations we choose the one that restores our curved model to linearity everywhere, the additive analysis of the transformed data will correspond exactly to the curved analysis of the original data. This reverse transformation cannot (in general) be expressed as an explicit algebraic function. Now it is generally understood that the method of unweighted least squares, formerly advocated for the estimation of polygene components of variance, is inefficient (and therefore misleading) and must

be replaced by weighted least squares or maximum likelihood. I submit that the arbitrary selection of scale must also be replaced by more accurate methods.

But in my opinion it is asking for trouble to subject second-degree statistics to any more searching analysis than, say, division into genetic and environmental components (and even this gives unsatisfactory results only too often). I consider that the more complicated analysis into components for dominance, epistacy and the like leads in practice only to self-deception or frustration. This certainly seems to be true at the present time, and the considerations mentioned above suggest that it will go on being true. If, as seems advisable, we restrict ourselves to a more modest analysis of means (using variances only as indicators of accuracy), it will be simpler to work in terms of the curved model. It may also make better biological sense, for the curved gene response curve is a combination of additive and multiplicative effects, whereas the reverse transformation must usually appear somewhat arbitrary. The data of Giesbrecht (1959), published in sufficient detail for us to try the effect of transformations, illustrate this point.

The estimates are:

	Days to silking		Days to pollen shedding	
	1954	1955	1954	1955
x	0·93	1·065	0·88	1·05
b	93·0	77·8	93·7	74·2
d_1	−1·0	−0·3	−1·8	−0·5
d_2	13·6	1·8	17·8	2·3
h_1	−6·5	−2·8	−7·7	−2·1
h_2	0	0·6	−0·1	0·6

It again appears that the analysis is capable of quite credible results, in that d_1 and h_2 do not come to much. Indeed, we might profitably set h_2 permanently zero. On the other hand, quite similar sets of data give rise to dissimilar estimates. The present situation is perhaps as over-flexible as the additive analysis is inflexible, so that inclusion of the F_3 is essential to steady the estimation. Now, as Giesbrecht points out, it is difficult to find a satisfactory transformation to linearity. In the case of 1954 'days to silking', for example, only one of the scaling tests fails. All would be well if the lower parental mean were $1\frac{1}{2}$ days earlier. It appears, therefore, that the required scale will be roughly linear except for a sharp drop at the lower end. When constants are fitted to this lower end, however, we find from the distributions quoted by Giesbrecht (p. 333) that a suitable scale would replace 1, 2, 3, 4, 5, 6, etc., by 0, 17, −6, 7, 5, 6, etc. The simplest polynomial transformation is equally unsatisfactory, for it rises and then falls again with time instead of steadily increasing. I have no doubt that a more sensible scale could be found; but it will not be found easily. Similar difficulties arise with Giesbrecht's other data, and indeed are likely to be common once the necessity for precise scaling is accepted. This new type of analysis therefore seems to offer some advantage in flexibility—and credibility—over the usual linear model. It is clear that a large experiment is

essential for the investigation of any polygenetic situation; no satisfactory approximation to reality can be obtained from half a dozen family means. This conclusion—scarcely surprising—suggests that such sophisticated analysis will always be too expensive for practical breeding work. The curvilinear analysis does, however, show some promise for strictly genetical purposes. Even if it turns out to be still insufficient to describe the complexities of real life, it can at least keep the mathematicians occupied.

SUMMARY

The usual conventions are relaxed to permit the introduction of a curved genetic model that shows some attractive features. Linear polygene analysis is examined in the light of this more flexible model. It is shown that great care is necessary in the choice of scale, since variances are more sensitive than means to small deviations from additivity. Inclusion of the F_3 is necessary for successful prediction by extrapolation. The genetical validity of any type of polygene analysis is discussed. The new model is quite promising for the analysis of means; but I think that the (more ambitious) analysis of variances is likely to remain intractable, for both genetical and statistical reasons.

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A further examination of the relative efficiency of three methods of selection for genetic gains under less-restricted conditions

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1. INTRODUCTION

In animal or plant breeding, three methods may be used for multi-character selection. These are tandem selection, independent culling levels and index selection (Fairfield Smith, 1936; Hazel & Lush, 1942; Hazel, 1943; Young & Weiler, 1960). Briefly tandem selection involves selection for several traits one at a time over a number of generations, while in the method of independent culling levels simultaneous but independent cullings are carried out on different traits. In index selection, each animal is given a score, which combines several traits, after weighting them according to their relative importance. Hazel & Lush (loc. cit.) compared the relative efficiency of these methods, in terms of genetic gain, when the traits under selection were independent and had equal heritability, economic weight and variance. They concluded that the index method was most efficient and tandem selection least, the efficiency of selection by independent culling levels being intermediate.

All the above methods have different applications in the field. Under some conditions, a method which is more efficient in terms of genetic gain may not be more efficient in terms of cost and labour. It is therefore important to know the amount by which one method is superior to another, in genetic gain, so that an optimal selection plan can be made. Since production traits in animals or plants are often correlated and have different values for heritability, variance and economic weight, it seems desirable to extend the comparisons of Hazel & Lush for more general conditions and to outline methods of comparing relative efficiency. The discussion has been confined to efficiency in terms of genetic gain in economic units, since problems of costs and labour will vary with circumstances.

As the three selection methods differ in principle, the relative efficiency of any two methods usually cannot be expressed in simple terms, and in the present work generalization has not always been possible. However, in all cases, numerical computations have been made to assess the extent to which changes in various pertinent factors affect relative efficiency. Comparisons involving the independent culling method, for correlated traits, are limited to the case of two characters, as the theory has not so far been extended to a greater number.

2. NOTATION

a_i	Economic weight of the ith trait.
b_i	Weighting coefficient for the ith trait in a selection index.
$bG_{j.i}$	Genetic regression coefficient of the jth trait on the ith trait $= \dfrac{G_{ij}}{G_{ii}}$.
C_{ij}	Elements of the inverse phenotypic variance and covariance matrix.
G_i	Genetic value of the ith trait.
G_{ij}	Genetic covariance of the ith and the jth traits (for variance $i = j$).
ΔG_i	Expected genetic change in the ith trait due to selection $= Ih_i^2\sqrt{(P_{ii})}$.
H_C	Expected total genetic gain in value by using the independent culling levels method. It is written as H'_C when traits under selection are independent.
H_T	Expected total genetic gain in value by using tandem selection. When traits are independent, H'_T is used.
H_X	Expected total genetic gain in value by using index selection. When traits are independent, H'_X is used.
h_i^2	Heritability for the ith trait.
h_{12}	$G_{12}/\sqrt{(P_{11}P_{22})}$.
I	Total selection differential in standardized units.
i_j	Selection differential for the jth trait in standardized units.
n	Number of traits under consideration.
$[P]$	Phenotypic variance and covariance matrix.
P_{ij}	Phenotypic covariance of the ith and jth trait (for variance $i = j$).
p	The total proportion of animals saved.
q_i	Proportion of animals saved with respect to the ith trait.
X_i	The ith trait.
x_i	Measurement of the ith trait.
z	The ordinate of the standardized normal curve at the point of truncation.
α_i, β_i	Constants used in the independent culling levels methods.
η, ξ	Total genetic change, in standardized units, in a trait, X_1 or X_2, due to simultaneous selection for both X_1 and X_2.
λ_i	Relative importance of the ith trait, $= a_i h_i^2\sqrt{(P_{ii})}$.
ρ	Phenotypic correlation coefficient.
ρ_G	Genetic correlation coefficient.

3. METHOD OF COMPARISON

(*a*) *Genetic gains by three methods of selection*

To compare the efficiency of tandem, independent culling and index selection methods, the genetic gain expected from each must be calculated. For the present purpose, the generation length within each class of livestock can be regarded as constant and the expected genetic gains per generation can be compared. On this basis, the expected genetic gains by tandem selection are the gains achieved by

single trait selection, although, for convenience, this will be referred to as gains by tandem selection in the subsequent discussion.

When using tandem selection, the expected gain per generation (H_T) by selection of the trait X_i can be shown to be

$$H_T = \Delta G_i\left(a_i + \sum_{j\neq i}^{n} a_j b_{G_{j\cdot i}}\right)$$

$$= Ih_i^2\sqrt{(P_{ii})}\left(a_i + \sum_{j\neq i}^{n} a_j b_{G_{j\cdot i}}\right) \qquad (1)$$

When X_i is independent of other traits the expected genetic gain H'_T is

$$H'_T = a_i I h_i^2 \sqrt{(P_{ii})}.$$

When the method of independent culling levels is used, the expected genetic gain per generation by simultaneous culling of two correlated traits X_1 and X_2 is

$$H_C = \{a_1\alpha_1\sqrt{(P_{11})} + a_2\beta_1\sqrt{(P_{22})}\}\, i_1 + \{a_1\alpha_2\sqrt{(P_{11})} + a_2\beta_2\sqrt{(P_{22})}\}\, i_2$$
$$= V_1 i_1 + V_2 i_2 \qquad \text{(Young \& Weiler, loc. cit.)} \qquad (2)$$

where i_1 and i_2 are the selection differentials of X_1 and X_2, when H_C is a maximum,

$$\alpha_1 = \frac{h_1^2 - \rho h_{12}}{1-\rho^2}, \qquad \alpha_2 = \frac{h_{12} - \rho h_1^2}{1-\rho^2},$$

$$\beta_1 = \frac{h_{12} - \rho h_2^2}{1-\rho^2}, \qquad \beta_2 = \frac{h_2^2 - \rho h_{12}}{1-\rho^2}.$$

When X_1, X_2 are independent, equation (2) reduces to

$$H'_C = a_1 h_1^2\sqrt{(P_{11})}\, i_1 + a_2 h_2^2\sqrt{(P_{22})}\, i_2, \qquad (3)$$

which is identical to the formula presented by Hazel & Lush (loc. cit.), for in this case

$$\alpha_1 = h_1^2, \qquad \alpha_2 = 0,$$
$$\beta_1 = 0, \qquad \beta_2 = h_2^2.$$

Equation (3) can be expanded to n independent traits, and the expected gain due to selection for n traits is

$$H'_C = \sum_{i=1}^{n} a_i h_i^2 i_i \sqrt{(P_{ii})}, \qquad (4)$$

where i_i may be written as $\frac{z_i}{q_i}$, where $\prod_{i=1}^{n} q_i = p$ the proportion of animals saved, and z_i is the ordinate of the normal curve corresponding to q_i.

Index selection was first used by Fairfield Smith (loc. cit.) for plants and later by Hazel (loc. cit.) in animal-breeding. The expected genetic gains by using the selection index may be written as (Fairfield Smith, loc. cit.; Morley, 1950)

$$H_X = I\sqrt{\left(\sum_{i=1}^{n} b_i^2 P_{ii} + 2\sum_{i\neq j} b_i b_j P_{ij}\right)}. \qquad (5)$$

When the traits under selection are independent the gain by index selection simplifies to

$$H'_X = I\sqrt{\left(\sum_{i=1}^{n} b_i^2 P_{ii}\right)} \tag{6}$$

(b) Ratios of relative efficiencies

In comparing the relative efficiency of the selection index and other methods it is necessary to consider the ratios H_X/H_T, H_X/H_C and H_C/H_T, or, in the case of independent traits, H'_X/H'_T, H'_X/H'_C and H'_C/H'_T.

To simplify the above ratios, we now consider the relationships between the different genetic gains. In an index, when the traits under selection are independent,

$$P_{ij} = G_{ij} = 0\ (i \neq j), \quad \text{so that} \quad b_i = a_i h_i^2.$$

If we write $\lambda_i = a_i h_i^2 \sqrt{(P_{ii})}$, then

$$H'_T = I\lambda_i, \tag{7}$$

$$H'_C = \sum_{i=1}^{n} i_i \lambda_i, \tag{8}$$

and

$$H'_X = I\sqrt{\left(\sum_{i=1}^{n} \lambda_i^2\right)}. \tag{9}$$

Using these relations, the ratio

$$\frac{H'_X}{H'_T} = \frac{I\sqrt{\left(\sum_{i=1}^{n} \lambda_i^2\right)}}{I\lambda_i} = \frac{\sqrt{\left(\sum_{i=1}^{n} \lambda_i^2\right)}}{\lambda_i}, \tag{10}$$

which is independent of selection intensity (this is true also for H_X/H_T). The ratio

$$\frac{H'_X}{H'_C} = \frac{I\sqrt{\left(\sum_{i=1}^{n} \lambda_i^2\right)}}{\sum_{i=1}^{n} i_i \lambda_i} \tag{11}$$

is dependent on selection intensity and the number of traits under selection as well as the relative values of λ_i, as is also the ratio

$$\frac{H'_C}{H'_T} = \frac{\sum_{i=1}^{n} i_i \lambda_i}{I\lambda_i} \tag{12}$$

Hence, in comparing the relative efficiency of these selection methods, when the traits under selection are independent, changes in relative efficiency of selection methods are functions of selection intensity (p), number of traits under selection (n) as well as the relative importance of the traits represented by the various λ values. An interesting point is that, when estimating the relative importance of any trait X_i, for a given set of parameters only the value of λ_i needs to be considered, as the components of λ_i (i.e. $a_i h_i^2$ and $\sqrt{(P_{ii})}$) have only the indirect importance of determining the size of λ_i.

When traits under selection are correlated, the relative efficiency is also affected by the levels of the phenotypic and genetic correlations (ρ and ρ_G). Therefore, in the following comparisons attempts have been made to relate changes in relative efficiency to changes in the five factors n, p, λ, ρ and ρ_G.

4. RESULTS

(a) *Effect of number of characters under selection and of selection intensity for equal λ-values*

The effects of number of traits under selection and selection intensity on the relative efficiency of selection methods have been investigated by Hazel & Lush (loc. cit.), and their formulae can be derived simply by the use of the ratios of genetic gains.

If traits under selection are uncorrelated and have equal λ-values,

$$\frac{H'_X}{H'_T} = \frac{\lambda_i \sqrt{n}}{\lambda_i} = \sqrt{n}, \tag{13}$$

$$\frac{H'_X}{H'_C} = \frac{I\lambda_i \sqrt{n}}{ni\lambda_i} = \frac{I}{i\sqrt{n}}, \tag{14}$$

$$\frac{H'_C}{H'_T} = \frac{ni\lambda_i}{I\lambda_i} = \frac{ni}{I}. \tag{15}$$

Hazel & Lush (loc. cit.) concluded that:

(i) The index is more efficient than tandem selection, the relative efficiency of the former increasing with n.

(ii) The index is more efficient than independent culling, its superiority rising with increasing n but falling with increasing selection intensity.

(iii) The independent culling method is more efficient than tandem selection, the superiority of the former rising both with increasing n and increasing selection intensity.

(b) *Effect of unequal λ-values*

When traits under selection have different λ-values, the ratios of genetic gains for uncorrelated traits cannot be reduced to simpler forms. To simplify computations, we now consider situations where n is fixed, since the effects of changes in n on the relative efficiency of various comparisons have been discussed earlier. It is also understood that in carrying out tandem selection, the trait with the largest λ-value is selected and the following calculations are based on this condition.

(i) *Index and tandem selection*

The relative efficiencies of the index and tandem selection for $n = 2$ and $n = 3$ are calculated according to (10). The results are shown in Table 1.

The relative efficiency varies with changes in relative importance of the traits. The superiority of the index method is at a maximum when the traits under selection have equal values of λ. This superiority decreases appreciably when λ-values are different. Thus, when $n = 2$, the index is 41% more efficient when $\lambda_1 = \lambda_2$, but

Table 1. *The relative efficiency of tandem and index selection for uncorrelated traits of unequal importance*

Two traits			Three traits							
λ_1*	λ_2	H'_X/H'_T†	λ_1	λ_2	λ_3	H'_X/H'_T	λ_1	λ_2	λ_3	H'_X/H'_T
1	1	1·41	1	1	1	1·73	2	2	1	1·50
2	1	1·12	2	1	1	1·22	3	2	1	1·25
3	1	1·05	3	1	1	1·11	3	3	1	1·45
4	1	1·03	4	1	1	1·06	4	3	1	1·28
5	1	1·02	5	1	1	1·04	5	3	1	1·18
6	1	1·01	6	1	1	1·03	6	4	1	1·21

* λ_i = relative importance of the ith trait = $a_i h_i^2 \sqrt{(P_{ii})}$.
† H'_X/H'_T = relative efficiency of index over tandem selection.

only 5% more efficient when $\lambda_1 = 3\lambda_2$. The decline in relative superiority of the index is appreciable from the point $\lambda_1 = \lambda_2$ to $\lambda_1 = 2\lambda_2$, but with further increase in the difference between λ-values the decline is slow.

The same general pattern occurs when $n = 3$. It can be seen from Table 1 that if the λ_i are not all equal, the index has the greatest superiority when they are nearest to equality, that is, when their values are 2:2:1 or 3:3:1. The overall superiority of the index method is higher when $n = 3$ than when $n = 2$. This is not surprising because, as has been shown earlier, the relative efficiency of the index over the tandem method increases with increasing values of n.

(ii) *Index and independent culling levels*

Before (11) can be calculated, it is necessary to estimate the expected genetic gains by each method. The calculation of H'_X, the genetic gains by index selection, is reasonably simple as is indicated by equation (6), but calculation of H'_C, the genetic gains by using the culling method, involves the maximization of H'_C for a number of combinations of p- and λ-values. The results of maximization, giving various q-values for each p at which H'_C is a maximum, for two independent traits under selection are shown in Table 2. Table 2, which is an extension of a table presented

Table 2. *The appropriate proportion saved for two traits of unequal importance, for maximal genetic gains*

		$p = 0{\cdot}8$†		$p = 0{\cdot}5$		$p = 0{\cdot}2$		$p = 0{\cdot}1$		$p = 0{\cdot}05$	
λ_1*	λ_2	q_1‡	q_2	q_1	q_2	q_1	q_2	q_1	q_2	q_1	q_2
1	1	0·89	0·89	0·71	0·71	0·45	0·45	0·32	0·32	0·22	0·22
2	1	0·81	0·99	0·53	0·94	0·24	0·82	0·14	0·71	0·09	0·56
3	1	0·80	1·00	0·51	0·99	0·22	0·91	0·11	0·91	0·06	0·83
4	1	0·80	1·00	0·50	1·00	0·20	1·00	0·10	1·00	0·06	0·91
5	1	0·80	1·00	0·50	1·00	0·20	1·00	0·10	1·00	0·05	1·00
6	1	0·80	1·00	0·50	1·00	0·20	1·00	0·10	1·00	0·05	1·00

* λ_i = relative importance of the ith trait = $a_i h_i^2 \sqrt{(P_{ii})}$.
† p = total proportion of animals saved.
‡ q_i = proportion saved from the ith trait.

by Hazel & Lush (loc. cit.), is not only useful for subsequent discussions here, but may be of value in practice when λ-values of the traits are known.

The expected genetic gains of H'_C and H'_X and the ratios of relative efficiency are shown in Table 3. The superiority of the index over independent culling levels is

Table 3. *The relative efficiency of index and independent culling levels for uncorrelated traits*

		†$p = 0{\cdot}8$	$p = 0{\cdot}5$	$p = 0{\cdot}2$	$p = 0{\cdot}1$	$p = 0{\cdot}05$
λ_1*	λ_2	H'_X/H'_C‡	H'_X/H'_C	H'_X/H'_C	H'_X/H'_C	H'_X/H'_C
1	1	1·19	1·18	1·12	1·10	1·09
2	1	1·11	1·09	1·08	1·07	1·07
3	1	1·05	1·05	1·05	1·05	1·05
4	1	1·03	1·03	1·03	1·03	1·03
5	1	1·02	1·02	1·02	1·02	1·02
6	1	1·01	1·01	1·01	1·01	1·01

* λ_i = the relative importance of the ith trait.
† p = proportion of animals saved.
‡ H'_X/H'_C = the relative efficiency of the index and independent culling levels.

again at a maximum when $\lambda_1 = \lambda_2$, and there is a steady decline in its superiority as the ratio λ_1/λ_2 increases. Thus when $\lambda_1 = 4\lambda_2$ the difference in relative efficiency is only small, irrespective of selection intensity. If the λ-values are fixed the index method is more efficient when selection intensity is low; this is consistent with results shown earlier. An interesting point is that the efficiency of the index relative to independent culling and to tandem selection is the same when $\lambda_1 = 3\lambda_2$ (Tables 1 and 3), because, in this situation, H'_C is a maximum when culling is done entirely on the more important trait, thus reducing the independent culling method to that of single character selection. An exception is that when p is small (0·05) some attention may be paid to the less important trait but the additional genetic gain through this is negligible.

(iii) *Tandem selection and independent culling levels*

The ratios of relative efficiency using values of H'_T and H'_C calculated earlier are shown in Table 4. The superiority of independent culling levels over the tandem

Table 4. *The relative efficiency of tandem selection and independent culling levels for uncorrelated traits*

		†$p = 0{\cdot}8$	$p = 0{\cdot}5$	$p = 0{\cdot}2$	$p = 0{\cdot}1$	$p = 0{\cdot}05$
λ_1*	λ_2	H'_C/H'_T‡	H'_C/H'_T	H'_C/H'_T	H'_C/H'_T	H'_C/H'_T
1	1	1·20	1·21	1·26	1·28	1·30
2	1	1·00	1·02	1·04	1·04	1·05
3	1	1·00	1·00	1·00	1·01	1·01
4	1	1·00	1·00	1·00	1·00	1·00
5	1	1·00	1·00	1·00	1·00	1·00
6	1	1·00	1·00	1·00	1·00	1·00

* λ_i = the relative importance of the ith trait.
† p = proportion of animals saved.
‡ H'_C/H'_T = relative efficiency of independent culling levels and tandem selection.

method is again at a maximum when $\lambda_1 = \lambda_2$ and, as in previous cases, there is a sudden decrease in relative efficiency of the superior method when $\lambda_1 > \lambda_2$. When $\lambda_1 = 3\lambda_2$ the two methods have identical efficiency at lower selection intensity; at very high intensity the independent culling level method is very slightly more efficient. The overall efficiency of independent culling levels is higher when selection is more intense, which is consistent with the results shown earlier.

(c) *Effect of correlation between traits*

The relative efficiency of selection methods can also be affected by the changes in both phenotypic (ρ) and genetic correlations (ρ_G) between traits under selection, since changes in correlations affect genetic gains. In the following consideration the number of traits under selection is limited to two, as only bivariate distributions have so far been considered.

(i) *Index and tandem selection*

$$\frac{H_X}{H_T} = \frac{I\sqrt{(b_1^2 P_{11} + b_2^2 P_{22} + 2b_1 b_2 P_{12})}}{\Delta G_1 (a_1 + a_2 b_{G_{2.1}})}.$$

Consider a case when $P_{11} = P_{22} = a_1 = a_2 = 1$, $h_1^2 = h_2^2$, so that $\lambda_1 = \lambda_2$.
It can be shown that

$$b_1 = a_1(C_{11}G_{11} + C_{12}G_{21}) + a_2(C_{11}G_{12} + C_{12}G_{22}),$$
$$b_2 = a_1(C_{21}G_{11} + C_{22}G_{21}) + a_2(C_{21}G_{12} + C_{22}G_{22}),$$

where C_{ij} are the elements of the inverse phenotypic variance and covariance matrix $[P]^{-1}$. Under this condition $C_{11} = C_{22}$, $G_{11} = G_{22}$, so that $b_1 = b_2$ independent of changes in P_{12} and G_{12}, the phenotypic and genetic covariances. We have, therefore,

$$\begin{aligned} H_X &= I\sqrt{(2b_1^2 + 2b_1^2 P_{12})} \\ &= Ib_1\sqrt{\{2(1+P_{12})\}}. \end{aligned} \tag{16}$$

Now $b_1 = (C_{11} + C_{12})(G_{11} + G_{12})$. (Since $a_1 = a_2 = 1$, $G_{11} = G_{22}$, $G_{12} = G_{21}$.) In the matrix $[P]^{-1}$ the elements C_{ij} are

$$C_{11} = \frac{P_{22}}{P_{11}P_{22} - P_{12}^2}, \qquad C_{22} = \frac{P_{11}}{P_{11}P_{22} - P_{12}^2}, \qquad C_{12} = \frac{-P_{12}}{P_{11}P_{22} - P_{12}^2},$$

and b_1 therefore may be written as

$$b_1 = \frac{(1-\rho)(G_{11} + G_{22})}{1-\rho^2} = \frac{G_{11} + G_{12}}{1+\rho} \quad (\text{since } P_{11} = P_{22} = 1), \tag{17}$$

and, from equation (16),

$$\begin{aligned} H_X &= \frac{I(G_{11} + G_{12})\sqrt{(2(1+\rho))}}{1+\rho} \\ &= I(G_{11} + G_{12})\sqrt{\frac{2}{1+\rho}} \end{aligned} \tag{18}$$

Now, under the same condition, the gain by tandem selection

$$H_T = I\left(a_1\frac{G_{11}}{\sqrt{(P_{11})}}+a_2\frac{G_{11}}{\sqrt{(P_{11})}}\cdot\frac{G_{12}}{G_{11}}\right)$$
$$= I(G_{11}+G_{12}), \quad \text{since } a_1 = a_2 = 1, P_{11} = P_{22} = 1. \tag{19}$$

We have, therefore,

$$\frac{H_X}{H_T} = \sqrt{\frac{2}{1+\rho}} \tag{20}$$

The relative efficiency of these two methods under this special condition is a function of the phenotypic correlation, independent of changes in the genetic correlation. Thus, when $\rho = 0{\cdot}5$ the index is about 15% more efficient, when $\rho = 0{\cdot}3$ the index is about 23% more efficient, and when $\rho = -0{\cdot}5$ the index is 100% more efficient. The reason for this is intuitively clear because in the calculation of the expected gain by single character selection the phenotypic correlation makes no contribution. When $\rho = 0$, then the index is $\sqrt{2}$ times more efficient, as was shown earlier. The superiority of the index thus increases with decreasing levels of phenotypic correlation.

Table 5. *The relative efficiency of tandem and index selection for two correlated traits for different values of various parameters, but with one trait twice as important as the other*

Case	Phenotypic correlation	Genetic correlation	H_X/H_T†
*(A) $a_1 = a_2 = 1$ $h_1^2 = 2h_2^2 = 0{\cdot}6$ $\sqrt{(P_{11})} = \sqrt{(P_{22})} = 1$	0·5	−0·4	1·02
		−0·2	1·00
		0·2	1·00
		0·4	1·00
	0·3	−0·2	1·00
		−0·1	1·01
		0·1	1·03
		0·2	1·04
‡(B) or (C) $a_1 = 2a_2 = 2$ $h_1^2 = h_2^2 = 0{\cdot}6$ $\sqrt{(P_{11})} = \sqrt{(P_{22})} = 1$	0·5	−0·4	1·09
		−0·2	1·02
		0·2	1·01
		0·4	1·04
	0·3	−0·2	1·00
		−0·1	1·01
		0·1	1.04
		0·2	1·06

* a_i = economic weight of the ith trait.
h_i^2 = heritability of the ith trait.
$\sqrt{(P_{ii})}$ = standard deviation of the ith trait.

† H_X/H_T = relative efficiency of the index method over the method of independent culling levels.

‡ The same results may be obtained by putting

$$a_1 = a_2 = 1, \quad h_1^2 = h_2^2 = 0{\cdot}6, \quad \sqrt{(P_{11})} = 2\sqrt{(P_{22})} = 2 \quad \text{(Case C)}.$$

Equation (20) could no longer hold if there were changes in relevant parameters. For example, consider the following situations:

(A) $a_1 = a_2 = P_{11} = P_{22} = 1$, $h_1^2 = 2h_2^2 = 0{\cdot}6$ so that $\lambda_1 = 2\lambda_2$,

(B) $a_1 = 2a_2 = 2$, $P_{11} = P_{22} = 1$, $h_1^2 = h_2^2 = 0{\cdot}6$ and $\lambda_1 = 2\lambda_2$,

(C) $a_1 = a_2 = 1$, $P_{11} = 4P_{22} = 4$, $h_1^2 = h_2^2 = 0{\cdot}6$ and $\lambda_1 = 2\lambda_2$.

The relative efficiencies of index and tandem selection, for various levels of genetic and phenotypic correlations, are shown in Table 5. From the examples shown in Table 5, it is seen that the relative efficiency changes with changes in both phenotypic and genetic correlations. However, in the situation defined above (i.e. $\lambda_1 = 2\lambda_2$), the effects of changes in either phenotypic or genetic correlations are relatively small, except perhaps in one case, where a difference of 9% in relative efficiency has resulted when a large positive phenotypic correlation is accompanied by a large negative genetic correlation. The overall superiority of the index in the present cases, as compared with that when $\lambda_1 = \lambda_2$, has decreased considerably, which is consistent with the earlier findings. Results in Table 5 also show that the effects of changes in levels of genetic correlation vary with the changes in other parameters, thus generalization on its effect when traits are of unequal importance does not seem possible.

(ii) *Index and independent culling levels*

$$\frac{H_X}{H_C} = \frac{I\sqrt{(b_1^2 P_{11} + b_2^2 P_{22} + 2b_1 b_2 P_{12})}}{V_1 i_1 + V_2 i_2},$$

where
$$V_1 = \{a_1\alpha_1\sqrt{(P_{11})} + a_2\beta_1\sqrt{(P_{22})}\},$$
$$V_2 = \{a_1\alpha_2\sqrt{(P_{11})} + a_2\beta_2\sqrt{(P_{22})}\}.$$

Consider the special case used previously; when $P_{11} = P_{22} = a_1 = a_2 = 1$, $h_1^2 = h_2^2$ so that $\lambda_1 = \lambda_2$ and $b_1 = b_2$ as has been shown earlier. Then

$$V_1 = \alpha_1 + \beta_1,$$
$$V_2 = \alpha_2 + \beta_2.$$

Since
$$h_{12} = \frac{G_{12}}{\sqrt{(P_{11}P_{22})}},$$

we have
$$h_{12} = G_{12},$$

so that
$$\alpha_1 = \beta_2, \quad \alpha_2 = \beta_1 \quad \text{and} \quad V_1 = V_2.$$

Also
$$V_1 = (\alpha_1 + \beta_1)$$
$$= \frac{G_{11} + G_{12}}{1 + \rho}. \tag{21}$$

From equations (17) and (21) we find that, under this special condition, $V_1 = V_2 = b_1 = b_2$, so that

$$\frac{H_X}{H_C} = \frac{I\sqrt{\{2(1+\rho)\}}}{i_1 + i_2}, \tag{22}$$

again independent of genetic correlations. The relative efficiency depends on the phenotypic correlation and the selection intensity. In cases where $V_1 = V_2$, the value of H_C is a maximum when $i_1 = i_2$, thus, when $\rho = 0$, equation (22) is reduced to (14) for $n = 2$.

Equation (22) could no longer hold if there are changes in relative values of P_{ij}, h_i^2, a_i, etc. Where all these parameters differ, the expression for H_X/H_C remains complex. Some computations were made, however, using a series of values for the parameters, and the results are presented in Table 6. Four cases were considered:

(A) $P_{11} = P_{22} = 1$, $h_1^2 = h_2^2 = 0{\cdot}6$, $a_1 = a_2 = 1$ so that $\lambda_1 = \lambda_2$ with varying levels of phenotypic correlation.

(B) $P_{11} = P_{22} = 1$, $h_1^2 = 2h_2^2 = 0{\cdot}6$, $a_1 = a_2 = 1$ so that $\lambda_1 = 2\lambda_2$ with varying levels of phenotypic and genetic correlations.

(C) $P_{11} = P_{22} = 1$, $h_1^2 = h_2^2 = 0{\cdot}6$, $a_1 = 2a_2 = 2$ so that $\lambda_1 = 2\lambda_2$.

(D) $P_{11} = 4P_{22} = 4$, $h_1^2 = h_2^2 = 0{\cdot}6$, $a_1 = a_2 = 1$ so that $\lambda_1 = 2\lambda_2$.

Table 6. *The relative efficiency of the index and independent culling levels methods of selection for two correlated traits*

Case	Phenotypic correlation	Genetic correlation	H_X/H_C† $p = 0{\cdot}1$	H_X/H_C† $p = 0{\cdot}5$
*(A)	0·5	any value	1·03	1·05
$a_1 = a_2 = 1$	0·3	any value	1·07	1·09
$h_1^2 = h_2^2 = 0{\cdot}6$	−0·5	any value	1·22	1·43
$\sqrt{(P_{11})} = \sqrt{(P_{22})} = 1$				
	0·5	−0·4	1·01	1·02
		−0·2	1·00	1·00
		0·2	1·00	1·00
(B)		0·4	1·00	1·00
$a_1 = a_2 = 1$	0·3	−0·2	1·00	1·00
$h_1^2 = 2h_2^2 = 0{\cdot}6$		−0·1	1·01	1·01
$\sqrt{(P_{11})} = \sqrt{(P_{22})} = 1$		0·1	1·03	1·03
		0·2	1·04	1·04
	0·5	−0·4	1·09	1·09
		−0·2	1·02	1·02
		0·2	1·01	1·01
(C)‡ or (D)		0·4	1·02	1·03
$a_1 = 2a_2 = 2$	0·3	−0·2	1·00	1·00
$h_1^2 = h_2^2 = 0{\cdot}6$		−0·1	1·01	1·00
$\sqrt{(P_{11})} = \sqrt{(P_{22})} = 1$		0·1	1·04	1·04
		0·2	1·05	1·06

* a_i = economic weight of the ith trait.
h_i^2 = heritability of the ith trait.
$\sqrt{(P_{ii})}$ = standard deviation of the ith trait.

† H_X/H_C = relative efficiency of the index method over the method of independent culling levels.

‡ The same results may be obtained by putting

$$a_1 = a_2 = 1, \quad h_1^2 = h_2^2 = 0{\cdot}6, \quad \sqrt{(P_{11})} = 2\sqrt{(P_{22})} = 2 \quad \text{(Case D)}.$$

The superiority of the index increases with decreasing value of phenotypic correlation when $\lambda_1 = \lambda_2$. The index is also more efficient when the selection intensity is low, which is, of course, expected from earlier discussions. The superiority of the index is only relatively small when $\lambda_1 = 2\lambda_2$. In this case the effect of the genetic correlation again changes with changes in other parameters, and generalization of its effect on relative efficiency does not seem possible. The relative efficiency for some cases in Table 6 is similar to that shown in Table 5 for the comparison of the index with tandem selection. This is because the values of V_1 in all cases are several times larger than V_2. When this is so, H_C is a maximum when only the more important trait is selected, and the method of independent culling levels becomes equivalent to single character selection.

(iii) *Independent culling levels and tandem selection*

From equations (19) and (21), when $a_1 = a_2 = 1$, $P_{11} = P_{22} = 1$, $h_1^2 = h_2^2$, and $i_1 = i_2$,

$$\frac{H_C}{H_T} = \frac{2i_1}{I(1+\rho)} \tag{23}$$

When $\rho = 0$, equation (23) is reduced readily to equation (15) for uncorrelated traits. The relative efficiency in this case is again independent of genetic correlation, but is affected by changes in phenotypic correlation and selection intensity. Thus when $p = 0{\cdot}1$ and $\rho = 0{\cdot}5$ the culling method is 12% more efficient; for the same p-values, if $\rho = 0{\cdot}3$ the culling method is 16% more efficient, and if $\rho = -0{\cdot}5$ it is 64% more efficient. Hence, the superiority of the culling method increases with decreasing values of ρ. The overall efficiency of the culling method is slightly lower when there is a reduction in selection intensity. When $\lambda_1 = 2\lambda_2$, the efficiencies of the two selection methods are similar, as discussed earlier.

5. DISCUSSION

In estimating the relative efficiency of selection methods the ideal way, of course, would be to calculate the expected genetic gains by each selection method, following the steps described earlier. In most cases, however, it is possible to reach some conclusion from the results presented above, without carrying out tedious computations. Thus, for example, when two negatively correlated traits of equal importance are under selection, the index method is the choice when selection intensity is low. If the selection intensity is high, then independent culling levels may be more appropriate because of the relative simplicity of operation. Again, if one trait is three times more important than another, then tandem selection is probably the choice and other factors, such as correlations and selection intensity, have but minor effects on relative efficiency. It follows, therefore, that the question of relative efficiency is only important when the traits have about equal importance, and a simple computation of λ-values for each trait would often give sufficient ground for the rejection of more sophisticated methods of selection for genetic gains.

In addition to considering maximum gains in economic terms, it is necessary in animal breeding to estimate the changes in means of various important traits in future generations, since if traits are correlated genetically any changes in one might be accompanied by a deterioration of others. Continued application of a single scheme of selection might, therefore, not be desirable. For example, use of an index for selecting fleece weight and crimps based on parameters given by Morley (1955) and economic weights by Dunlop & Young (1960) would lead to an expected decrease in crimp number in future generations. If long-term decreases in crimps are undesirable in some circumstances, then it is important to alter the selection method or to select for fleece weight while at the same time controlling crimp number.

The method of selecting for one trait while keeping the other trait constant, using a specially constructed index, has been described by Morley (1955). However, it is worth noting that independent culling levels can also be applied in this way. From equations shown by Young & Weiler (loc. cit.), the genetic gains by the culling method for two correlated traits are

$$H_C = a_1 \sqrt{(P_{11})}\,\xi + a_2 \sqrt{(P_{22})}\,\eta,$$

where ξ and η are the expected genetic changes in traits X_1 and X_2 respectively. If we wish to keep $\eta = 0$, we have

$$\beta_1 i_1 + \beta_2 i_2 = 0,$$

$$i_2 = \frac{-\beta_1}{\beta_2} i_1 \tag{24}$$

For any given proportion of animals saved we can obtain a combination of i_1 and i_2 which satisfies (24). Again we have a choice of two methods of selection.

On the other hand, if it is desirable to make as much progress on one particular trait as possible, then a selection index can be used. Thus if X_1 and X_2 are two correlated traits, the aim is to construct an index in order to improve X_1 while at the same time using X_2 for correcting the environmental effects on X_1. This particular application of the index has been suggested by Hazel (loc. cit.) and a special case has been discussed by Rendel (1954), who found that the phenotypic regression coefficient of X_1 on X_2 might be used to advantage if the genetic correlation between X_1 and X_2 were smaller than the phenotypic correlation. However, in cases where the estimate of genetic correlation is available, it seems appropriate to apply an index which can account for the genetic covariance.

Suppose X, Y, Z are three traits in an animal and we wish to maximize the genetic changes in X. If we take one pair of traits, X, Y, or X, Z, at a time and denote X by X_1 and Y or Z by X_2, then an index

$$S = b_1 X_1 + b_2 X_2$$

can be formed. Now if we put $b_1 = 1$ and give no economic weight to X_2,

$$b_2 = \frac{P_{11} G_{21} - P_{12} G_{11}}{P_{22} G_{11} - P_{12} G_{21}}. \tag{25}$$

The expected genetic change in X_1 by using an index has been shown by Fairfield Smith (loc. cit.) and Morley (1950) to be

$$\Delta G'_X = I \frac{\sum_j b_j G_{ij}}{\sigma_S},$$

where σ_S is the standard deviation of the selection index. When $b_1 = 1$,

$$\Delta G'_X = I \frac{G_{11}}{\sqrt{(P_{11})}} \left(\frac{1 + b_2 \frac{G_{12}}{G_{11}}}{\sqrt{\left(1 + b_2^2 \frac{P_{22}}{P_{11}} + 2b_2 \frac{P_{12}}{P_{11}}\right)}} \right) \tag{26}$$

Remembering that the genetic change in X_1 by single character selection is

$$\Delta G = I \frac{G_{11}}{\sqrt{(P_{11})}},$$

equation (26) may be written as

$$\begin{aligned} \Delta G'_X &= \Delta G \left(\frac{1 + b_2 \frac{G_{12}}{G_{11}}}{\sqrt{\left(1 + b_2^2 \frac{P_{22}}{P_{11}} + 2b_2 \frac{P_{12}}{P_{11}}\right)}} \right) \\ &= \Delta G . K \text{ (say)}. \end{aligned} \tag{27}$$

The value of K gives information about the additional gain in X_1, in terms of percentages, when such an index is used. For example, a value of 1·20 for K indicates that 20% more gain can be expected by using such an index. It follows that the size of K indicates whether the index approach is worthwhile.

If the genetic covariance is zero,

$$b_2 = -\frac{P_{12}}{P_{22}} \tag{28}$$

and

$$\Delta G'_X = \Delta G . \frac{1}{\sqrt{(1 - \rho^2)}}.$$

Since ρ cannot be greater than unity, $\Delta G'_X \geqslant \Delta G$ ($\rho < 1$) and the index is more efficient when ρ is large. In these circumstances, the method suggested by Rendel (loc. cit.) has the same efficiency as the index. Now if the K value obtained by using Y as environmental correction for X is greater than that when Z is used, then, of course, Y should be chosen for this purpose. Following the same principle, an index can also be constructed using both Y and Z as environmental corrections for X, but the computations become tedious and the additional advantage is probably small.

Independent culling levels can also be used to this end by putting the economic weight of Y (or Z) equal to zero. Then we have

$$\begin{aligned} \Delta G'_X &= \{\sqrt{(P_{11})}\, \alpha_1\} i_1 + \{\sqrt{(P_{11})}\, \alpha_2\} i_2 \\ &= T'_1 i_1 + T'_2 i_2 \text{ (say)}, \end{aligned} \tag{29}$$

and $\Delta G'_X$ can be maximized accordingly. Here two alternative methods for increasing the genetic progress in a single trait, beyond that which may be achieved by single character selection, are available. The relative merit of these methods for the present purpose depends on the relative sizes of $\Delta G'_X$ which may be attained by each method, and numerical calculation in each case is necessary.

The relative efficiency of selection methods therefore may be affected by some special applications of these methods, apart from the various factors (n, p, etc.) discussed earlier. These special applications are often useful in animal-breeding work, and the two cases outlined above show some of the possibilities. However, the relative efficiency of any special applications of selection methods can probably be compared by the same principle used in this work, i.e. by comparing the relative genetic gains.

However, it seems appropriate to reiterate that the relative efficiency of selection methods in terms of genetic gains in economic value is but one of the several factors in determining an optimum method of selection. Factors such as practicability, costs, and the consequences due to the use of any method could all have significant bearing in choosing an optimum method.

It should be pointed out that the comparisons discussed in this work have been limited to linear cases: i.e. all the regressions involved are assumed to be linear. When this condition is altered, the relative efficiency of selection methods may be quite different.

SUMMARY

The relative efficiency of three methods of selection (index, independent culling levels and tandem) is compared in terms of genetic gains in economic units. The comparison covers cases where variances, heritabilities and economic weights are unequal, while the case of two correlated characters is also examined. Various factors may influence the relative efficiency, including selection intensity, the number of traits under selection, the relative importance of those traits (in terms of a factor λ, which is the product of economic weight, heritability and phenotypic standard deviation), and the correlations between them.

The conclusions are:

(i) In all circumstances the index is never less efficient than independent culling levels, though in some cases it is no more efficient. Independent culling is, in turn, never less, but in some cases no more efficient than tandem selection.

(ii) The superiority of the index over other methods increases with an increasing number of traits under selection, but decreases with increasing differences in relative importance, its superiority being at a maximum when the traits are of equal importance. The superiority of the index over independent culling levels decreases with increasing selection intensity, but its superiority over tandem selection is independent of intensity.

(iii) The superiority of independent culling over tandem selection increases with increasing selection intensity or an increasing number of traits under selection, but decreases with increasing differences in relative importance.

(iv) The relative efficiency of the index over other methods is much affected by the phenotypic correlation between traits when the traits are of equal importance, the relative efficiency of the index being higher when the phenotypic correlation is low or negative. The effect of genetic correlation is only apparent when the traits are of unequal importance and its influence on relative efficiency changes with changes in other parameters.

(v) The relative efficiency of selection methods may be changed by their use for special purposes.

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Tipsy, a new mutant in linkage group VII of the mouse

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The large collection of inherited locomotory disorders now known in the mouse can be placed roughly into three main categories: (i) the waltzer-shaker mutants, of which at least thirteen independent members are now known (Grüneberg, 1956), (ii) a group including dilute lethal, jimpy, jittery and Trembler, in which the syndrome typically includes convulsions, and (iii) mutants which walk unsteadily, often with a tremor or swaying movements, which may or may not be succeeded by ataxia, but without convulsions. Eight conditions of this type have already been described and the tipsy mutant is clearly another member of this last group. The present paper describes its abnormal behaviour and discusses its linkage relationships. Preliminary data on the inheritance and linkage relations of tipsy have been reported by Carter (1957, 1958) in private communications.

ORIGIN AND DESCRIPTION

Tipsy mice first appeared in 1956 among the F_2 offspring of a male carrying simultaneous mutations at the dilute and short-ear loci, which was one of the control offspring in a 37·5 r chronic gamma irradiation specific locus experiment. This male had been outcrossed to females A and B, F_1 offspring from a strain C3H/H × 101/H mating. Out of eight F_1 matings from A's progeny, two segregated for tipsy. With B, however, none out of five F_1 matings segregated, neither did any of six matings between A progeny and B progeny. Thus the mutant gene was probably derived from female A.

Tipsy mice can be distinguished from normals with some certainty at 6–8 days of age, when youngsters start active crawling. Instead of moving the hind-limbs alternately, the mutant flexes and extends both limbs together, producing what is best described as a 'rabbit gait'. About 4 days later a second characteristic defect appears, namely a tendency for the front part of the body to sway from side to side. There is marked variation with respect to this character. In severely affected animals there is an almost continuous rhythmic swaying, which leads to a reeling gait. The mutant takes an S-shaped course, first staggering over in one direction and then in the other, and occasionally falling over on its side. In other tipsy mice there is just an occasional side-to-side sway, with no obvious precipitating factor. The tendency to sway grows less in older adult mutants, disappearing completely in some. The rabbit-like gait, however, continues throughout life but is often interspersed at all ages with more or less normal locomotion. Mallyon (1953) reported

finding mice with rabbit gait in a stock carrying Fused (*Fu*), but it is clear from his description that a different entity is involved.

Tipsy mice can swim normally and are not deaf. When young ones are turned over on their back, however, they take decidedly longer than normals to right themselves. Moreover, when put down after being held up by the tail and gyrated, tipsy mice show an exaggerated sway in the direction of rotation and back again. The postural reaction when suspended by the tail (as described by Lyon, 1951) seems normal in tipsies; but their landing reaction, when held by the tail and quickly lowered towards a solid surface, is sometimes poorly developed.

SEGREGATION AND LINKAGE

The tipsy character is due to a fully recessive gene with 100% penetrance. Homozygotes of both sexes are fertile and when crossed have produced 86 tipsy and no normal offspring. Back-crosses of heterozygotes gave 49 tipsy and 52 normal, in good agreement with expectation. The symbol *ti* has been given to the gene concerned.

Crosses were made to the following mutant stocks for linkage tests: (i) dilute, short-ear (*d se*), (ii) White, Caracul, Brachyury (*Mi^{wh} Ca T*), (iii) Viable dominant spotting, Ragged, Rex (*W^v Ra Re*), with or without Danforth's short-tail (*Sd*). The results (Table 1) show that tipsy is fairly closely linked to Rex. Rex-tipsy recombination frequencies in the two sexes do not differ significantly and figures can therefore be combined, giving an overall frequency of $20{\cdot}23 \pm 1{\cdot}52\%$. Using Kosambi's (1944) transformation, this corresponds to a *Re–ti* map-distance of 21·45.

A three-point linkage test was then carried out to determine the position of *ti* with respect to other linkage group VII loci, namely vestigial (*vt*) and waved-2 (*wa-2*). When $\frac{+\ +\ +}{ti\ vt\ wa\text{-}2}$ females were crossed to males homozygous for all three recessive genes, offspring of the following phenotypes were obtained:

+	*ti*	*vt*	*wa-2*	*ti vt*	*ti wa-2*	*vt wa-2*	*ti vt wa-2*	total
258	26	4	63	66	1	23	164	605

ti shows $8{\cdot}93 \pm 1{\cdot}16\%$ recombination with *vt* and $29{\cdot}42 \pm 1{\cdot}85\%$ with *wa-2*, while *vt* and *wa-2* show $22{\cdot}15 \pm 1{\cdot}69\%$ recombination. So the order of loci must be *ti..vt..wa-2*. Further, since *Re* shows 21·7% recombination with *vt* (Michie, 1952) and *ti* shows only 9%, *ti* must lie between *Re* and *vt*.

DISCUSSION

Table 2 summarizes data on linkage relationships in group VII. In addition, Wright (1947) reported recombination frequencies in excess of 50% between *sh-2*, *wa-2* and sex. The results given in Table 2 are in fairly good agreement with each other, apart from those of Dickie (1955) on Alopecia (*Al*). Dickie found that Alopecia behaved in a very anomalous manner in linkage experiments, showing apparent linkage with members of several linkage groups; she discusses possible reasons for

Table 1. *Tests for linkage between tipsy* (ti) *and other mutants* (m)

Linkage group	Heterozygous parent	Phenotypes of progeny					Recombination frequency (%)
		m +	+ *ti*	+ +	*m ti*	Total	
II	$\frac{d +}{+ ti}$ ♂	46	39	43	48	176	53·2 ± 3·1
	$\frac{+ +}{d \ ti}$ ♂	18	32	19	20	89	
	$\frac{d +}{+ ti}$ ♀	31	33	36	33	133	51·9 ± 4·3
III	$\frac{W^v +}{+ \ ti}$ ♂	42	61	40	35	178	42·1 ± 3·7
	$\frac{W^v +}{+ \ ti}$ ♀	6	8	8	6	28	50·0 ± 9·4
V	$\frac{Ra +}{+ \ ti}$ ♂	41	37	35	44	157	50·3 ± 4·0
	$\frac{Ra +}{+ \ ti}$ ♀	15	12	14	11	52	48·1 ± 6·9
	$\frac{Sd +}{+ \ ti}$ ♂	15	25	10	17	67	40·3 ± 6·0
	$\frac{Sd +}{+ \ ti}$ ♀	6	8	11	5	30	53·3 ± 9·1
VI	$\frac{Ca +}{+ \ ti}$ ♂	25	19	29	28	101	56·4 ± 4·9
VII	$\frac{Re +}{+ \ ti}$ ♂	102	144	36	33	315	20·9 ± 2·2
	$\frac{Re \ ti}{+ +}$ ♂	3	2	13	21	39	
	$\frac{Re +}{+ \ ti}$ ♀	79	72	24	19	194	19·5 ± 2·1
	$\frac{Re \ ti}{+ +}$ ♀	16	8	60	65	149	
IX	$\frac{T +}{+ ti}$ ♂	24	25	30	22	101	51·5 ± 5·0
XI	$\frac{Mi^{wh} +}{+ \ \ ti}$ ♂	28	29	26	18	101	43·6 ± 4·9

this. Subsequent work by Dickie and colleagues suggests that *Al* is in fact closely linked to *Re*, a figure of 2% recombination between these loci being given by Green & Dickie (1959) in their linkage map of the mouse. Mrs P. W. Lane informs me that latest results from linkage tests now in progress, based on a later final classification for *Al*, give 6·7% recombination between *Re* and *Al*, and 21·7% between *Al* and *sh-2*.

Taken together, the data of Table 2 suggests the following map of linkage group VII (map-distances calculated by means of Kosambi's formula):

Re..7..*Al*....`2....*ti*..3..*Tr*..2..*sh-2*..2..*vt*.....25.....*wa-2*

Table 2. *Data on recombination percentages between loci in linkage group VII (sexes combined)*

Authors	Results
Falconer (1947)	*Re*.....20.....*sh-2*
	Re.....41.....*wa-2*
Snell *et al.* (see Grüneberg, 1952)	*sh-2*.....29..... *wa-2*
Carter (1951)	*Re*.....42.....*wa-2*
Carter & Phillips (1953)	*Re*.....19.....*sh-2*.....24..... *wa-2*
	Re.....44.....*wa-2*
Michie (1952)	*Re*....22....*vt*..2..*sh-2**
Michie (1955)	*vt*.....28..... *wa-2*
Falconer & Sobey (1953)	*Re*....23....*Tr*..3..*sh-2*
Dickie (1955)	*Re*.....33.....*Al*.....39.....*wa-2*
	Al.....28...*sh-2*
	Al.....45...*vt*
Lane (unpublished, 1960)	*Re*.....7.....*Al*.....22...*sh-2*
Searle (this paper)	*Re*.....20...*ti*..9...*vt*.....22..... *wa-2*

* Not known whether *sh-2* lies to right or left of *vt*.

This is similar to the map given by Green & Dickie (1959), though they show the distances in terms of recombination percentages. The close proximity of four genes, three with neurological actions, in the middle of the linkage group is worth noting. As Falconer & Sobey (1953) pointed out, this region may be one of low chiasma frequency. The exact order of these four loci is still not known for certain, though that given seems the most probable one. It might also be noted that Kosambi's formula, when applied to recombination percentages between distant loci, seems here to lead to an overestimate of map-distance. Thus estimates based on the *Re–wa-2* recombination frequencies alone would suggest these loci are over 60 map units apart, which is much higher than that obtained when intermediate loci are used.

In its behaviour, the tipsy mouse has some anomalous features in common with other mutants showing locomotor instability, but closely resembles none of them. Allelism with these mutants is generally ruled out by linkage relationships. Reeler (Falconer, 1951) and waddler (Yoon, 1959) mice sway and sometimes fall over, but the hindquarters are affected rather than the fore-parts of the body. Reelers, moreover, are sterile and show signs of mental deficiency. Ducky (Snell, 1955), vacillans (Sirlin, 1956) and agitans (Hoecker *et al.*, 1954) mice all tend to sway and fall over too, but they have a duck-like waddling gait different from the rabbit-like locomotion of tipsy. In ataxia (Lyon, 1955), Wabbler-lethal (Dickie *et al.*, 1952) and quivering (Yoon & Les, 1957) the effects on locomotion are much more severe than in tipsy, the mice becoming more or less completely helpless, with early death.

SUMMARY

Tipsy (symbol *ti*) is a fully penetrant recessive mutant in linkage group VII of the mouse. Homozygotes have a peculiar rabbit-like gait from the age of a week,

followed soon after by a tendency for the fore-part of the body to sway from side to side, leading to a reeling locomotion with falling over in severely affected mice. There is marked variation in manifestation and some amelioration in older animals; both sexes are fertile.

Linkage relationships in group VII are discussed and tipsy is compared with other mutants showing locomotor instability.

Data on tipsy's linkage relationships, etc., were collected by Miss Anne Constantine for Dr T. C. Carter, who later handed the material over to me. I am very grateful to both of them. I am also grateful to Dr Mary Lyon for suggesting improvements to the manuscript, and to Mrs Priscilla Lane for allowing me to refer to her unpublished linkage data.

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Natural selection as the process of accumulating genetic information in adaptive evolution*

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INTRODUCTION

Modern genetic studies have shown that the instructions for forming an organism are contained in the nucleus of the fertilized egg. In the language of information theory, we may say that in the process of development the genetic (hereditary) information of an organism is transformed into its phenotypic (organic) information. Thus, to account for the tremendous intricacy of organization in a higher animal, there must exist a sufficiently large amount of genetic information in the nucleus.

What is the origin of such genetic information? If the Lamarckian concept of the inheritance of acquired characters were accepted, one might be justified in saying that it was acquired from the environment. However, since both experimental evidence and logical deductions have entirely failed to corroborate such a concept, we must look for its source somewhere else.

We know that the organisms have evolved and through that process complicated organisms have descended from much simpler ones. This means that new genetic information was accumulated in the process of adaptive evolution, determined by natural selection acting on random mutations.

Consequently, natural selection is a mechanism by which new genetic information can be created. Indeed, this is the only mechanism known in natural science which can create it. There is a well-known statement by R. A. Fisher that 'natural selection is a mechanism for generating an exceedingly high degree of improbability', owing to which, as will be seen, the amount of genetic information can be measured. It may be pertinent to note here that the remarkable property of natural selection in realizing events which otherwise can occur only with infinitesimal probability was first clearly grasped by Muller (1929).

The purposes of the present paper are threefold. First, a method will be proposed by which the rate of accumulation of genetic information in the process of adaptive evolution may be measured. Secondly, for the first time, an approximate estimate of the actual amount of genetic information in higher animals will be derived which might have been accumulated since the beginning of the Cambrian epoch (500 million years), and thirdly, there is a discussion of problems involved in the storage and transformation of the genetic information thus acquired. There is a vast field

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of fundamental importance which awaits the fruitful activities of statisticians and other applied mathematicians collaborating with biologists.

THE CONCEPT OF A SUBSTITUTIONAL LOAD

A unit process in adaptive evolution is the replacement in a Mendelian population of one allele by another which is better fitted to a new environment. It was pointed out by Haldane (1957) that if this is carried out by premature death of less fit individuals, it may cost a number of deaths equal to about thirty times the population number. I proposed the term substitutional (or evolutional) load to express the decrease of population fitness (in the Darwinian sense) in the process of such a gene substitution (Kimura, 1960*a*, *b*).

Let us consider the simplest situation in which the population consists of haploid organisms, such as some fungi. In such a case, each gene exists in a single dose in a somatic cell. Let x be the frequency (relative proportion) of a gene A which is in the process of being substituted for its allele A' because of its selective advantage over A'. Then the rate of change in gene frequency x is given by

$$\frac{d}{dt}\log x - \frac{d}{dt}\log(1-x) = s,$$

or

$$\frac{dx}{dt} = sx(1-x),$$

where s (>0) is the selective advantage measured in Malthusian parameters (Fisher, 1930), i.e. in terms of its contribution to the geometric growth-rate of the population, and t is the time.

Since the population at a given moment contains the unfit genotype A' in the proportion of $1-x$, the total decrease in population fitness, also measured in Malthusian parameters, throughout the process of substitution is

$$L = \int_0^\infty s(1-x)\,dt = \int_p^1 s(1-x)\frac{dx}{sx(1-x)} = \int_p^1 \frac{dx}{x} = -\log_e p,$$

where p is the initial value of x. Thus we have

$$L = -\log_e p. \tag{1}$$

This is the expected substitutional load for a haplont if the substitution proceeds at the rate of one gene per generation. The actual substitutional load may be obtained by summing the above quantity over all relevant gene loci, each weighted according to the rate of substitution per locus per generation.

$$L_e = \sum \epsilon L = -\sum \epsilon \log_e p, \tag{2}$$

where ϵ is the rate of substitution per locus.

The situation is much more complicated for higher animals and plants, in which each gene exists in double dose within a somatic cell (diploidy) and gene interaction

within a locus (dominance) becomes important. It can be shown (cf. Kimura, 1960*b*) that, if the selective advantages of the genotypes AA and AA' over $A'A'$ are s and sh respectively, then the load produced by substituting A for A' is

$$L = -\frac{1}{h}\left\{\log_e p + (1-h)\log_e \frac{1-h}{h+(1-2h)p}\right\}, \qquad (s \geqq 0,\ 1 \geqq h \geqq 0) \qquad (3)$$

where p is the initial frequency of A in the population and h represents the degree of dominance of A over A' in fitness. One salient feature of this result is that L does not depend directly on s, the magnitude of selective advantage involved.

In Fig. 1(*a*) and (*b*), values of L are plotted for various values of h and p. It may

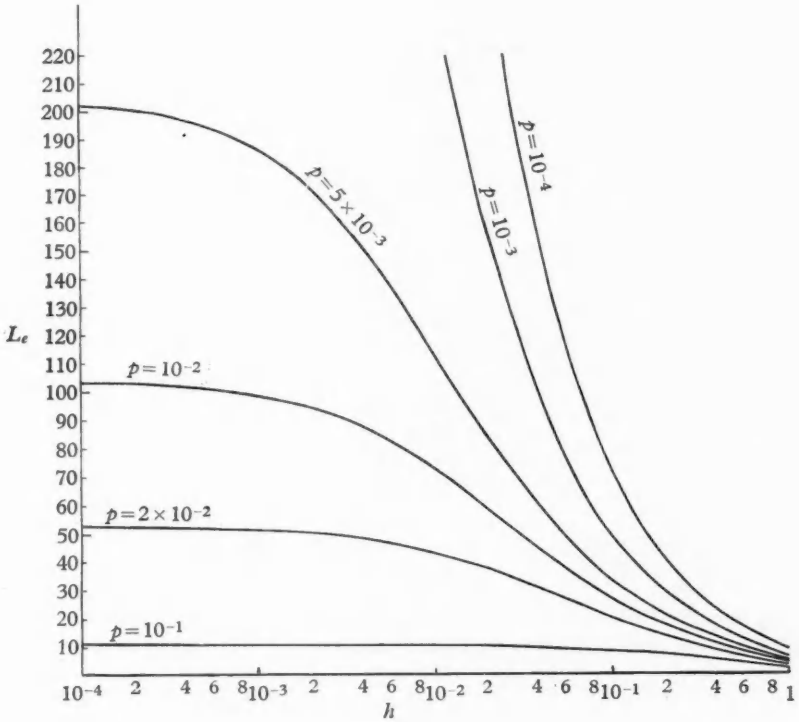

Fig. 1*a*.

be seen from this figure that L increases as p decreases, while it decreases as h increases. In man, the typical frequency of 'recessive' deleterious genes is of the order of 1%, and if we assume that their dominance in fitness is about 2% as in recessive lethals of the fruit-fly *Drosophila melanogaster*, L turns out to be about 59.

As in the case of the haplonts, the substitutional load is given by

$$L_e = \sum \epsilon L = -\sum \frac{\epsilon}{h}\left\{\log_e p + (1-h)\log_e \frac{1-h}{h+(1-2h)p}\right\}. \qquad (4)$$

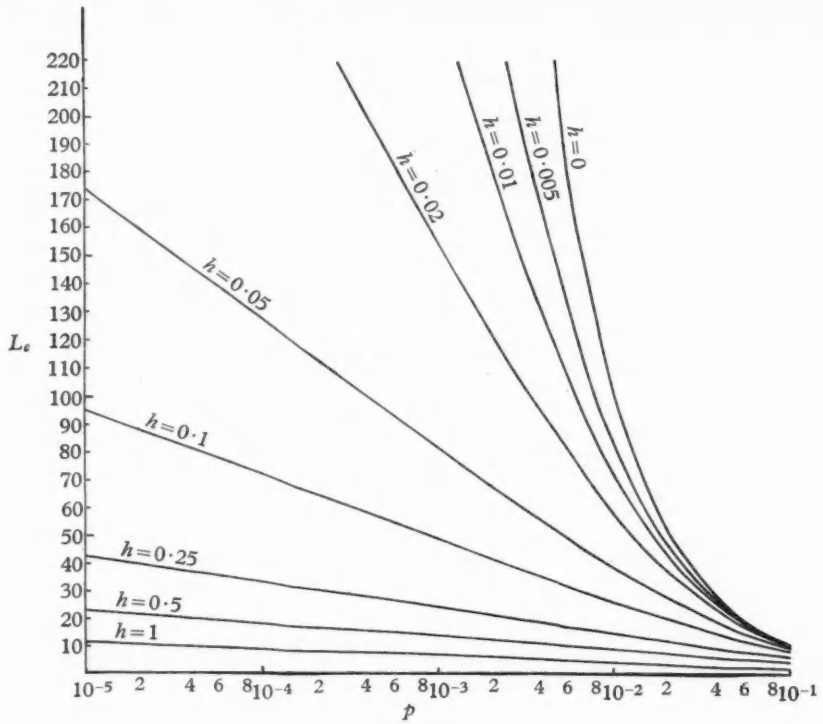

Fig. 1*b*.

Fig. 1 (*a*) and (*b*). Graphs showing the substitutional load L as a function of initial gene frequency p and degree of dominance h. L is the decrease in fitness which is expected if the gene substitution proceeds at the rate of one gene per generation.

THE SUBSTITUTIONAL LOAD AS A MEASURE OF GAIN IN GENETIC INFORMATION

I now propose to show that the rate of accumulation of genetic information denoted by H is directly proportional to the substitutional load, namely,

$$H = \frac{L_e}{\log_e 2} \approx 1{\cdot}44 L_e \quad \text{bits per generation,} \tag{5}$$

where bit is a commonly used unit of the amount of information equivalent to the information content of choosing between a pair of alternatives, say 0 or 1, with equal probability (0·5). The above relation may be derived from two independent courses of reasoning:

(1) If those individuals which are to be eliminated by natural selection in the process of progressive evolution were kept alive and allowed to reproduce at the same rate as the favoured individuals, the population number would become, after t generations,

$$e^{L_e t}$$

times its initial value. This means that natural selection allows an incident to occur with probability one, which, without selection, could occur only with a probability of

$$1/e^{L_e t} = e^{-L_e t}.$$

Thus information gained through t generations amounts to

$$-\log_2 e^{-L_e t} = \frac{L_e t}{\log_e 2} \text{ bits},$$

and therefore information gained per generation is

$$H = \frac{L_e}{\log_e 2} = (1{\cdot}442\ldots) L_e \text{ bits},$$

as was to be shown.

(2) Consider a population of haploid organisms. Let p be the initial frequency of an advantageous gene A. The probability that the gene A is ultimately established in the population is 1 under natural selection, while it is only p if natural selection were not working and the fixation of genes were left to the action of random genetic drift. Thus the amount of information corresponding to this gene substitution is

$$H = \log_2 \frac{1}{p} = -\log_2 p \text{ bits}.$$

On the other hand, we have shown that for one-gene substitution

$$L = -\log_e p. \qquad \text{(cf. (1))}$$

Therefore

$$H = -\log_2 e^{-L} = \frac{L}{\log_e 2}.$$

ESTIMATION OF THE INFORMATION GAIN IN THE ACTUAL PROCESS OF EVOLUTION

As shown above, the gain of genetic information is directly proportional to the substitutional load, and the problem of estimating the former (H) is now reduced to that of estimating the latter (L_e). However, since evolution is usually an exceedingly slow process in comparison to our ordinary life-span, it may be very difficult to determine L_e from direct observation. Haldane (1949) has shown, based on paleontological data of Simpson (1944), that the standard rate of evolution in morphological characters is of the order of one-tenth of a darwin, one darwin standing for a change by a factor of e per million years.

In an attempt to derive theoretically some fundamental genetic parameters such as mutation rate (μ) and degree of dominance (h) from the standard rate of evolution in the past, I proposed what I called the principle of minimum genetic load (Kimura, 1960*b*), a hypothesis that in the process of evolution the genetic parameters tend to be adjusted such that the total genetic load is minimized. In particular,

$$L_T = L_e + L_m$$

may be minimized in adaptive evolution. Here L_m stands for the mutational load which arises through the elimination of deleterious genes produced by recurrent mutation (cf. Crow, 1958). Based on this principle, it was demonstrated that the spontaneous mutation rate per gamete, $\Sigma\mu$, and the harmonic mean of the degree of dominance of mutant genes in fitness, $\bar{h}$, can be derived from the rate of substitution of genes and the total amount of hidden deleterious effect, per gamete, of mutant genes:

$$\left.\begin{aligned} \Sigma\mu &= \frac{0{\cdot}3419E}{\bar{h}}(1+1{\cdot}720\bar{h}+\ldots), \\ \bar{h} &= 0{\cdot}6838\sqrt{\frac{E}{2D}}\left(1+1{\cdot}018\sqrt{\frac{E}{2D}}+\ldots\right), \end{aligned}\right\} \tag{6}$$

where E is the rate of substitution of genes in horotelic evolution (standard rate evolution, cf. Simpson, 1944).

$$E = \Sigma\epsilon,$$

and D is the total amount of genetic damage per gamete expressed in lethal equivalents (cf. Morton, Crow & Muller, 1956). If we take $E = \frac{1}{300}$, an approximate value suggested by Haldane (1957), and $D = 2$, the one obtained by Morton, Crow & Muller from the study of inbreeding in man, we get

$$\bar{h} = 0{\cdot}0203 \quad \text{and} \quad \Sigma\mu = 0{\cdot}0581,$$

both of which agree fairly well with the corresponding observed values in the fruit-fly *Drosophila* ($\bar{h}$ about 2%, $\Sigma\mu$ about 4%). This is remarkable since the calculation is based on a simplified assumption that evolution has proceeded at a constant rate over an indefinitely long time. The calculation also supplies, at the same time, the substitutional and mutational loads:

$$L_e = 0{\cdot}206, \qquad L_m = 0{\cdot}099.$$

I will take $L_e = 0{\cdot}2$ and $L_m = 0{\cdot}1$ for the present purpose.

Then, the rate of accumulation of genetic information becomes approximately

$$H = 0{\cdot}29 \text{ bits per generation,}$$

if we apply relation (5). Similarly, we may calculate the amount of information gained by eliminating deleterious mutant genes by using the relation $H = L_m/\log_e 2$. This is an amount which exactly cancels out the loss of information by mutation. Table 1 is a balance-sheet of genetic information in evolution.

Table 1. *Balance sheet of genetic information in the process of horotelic evolution (bits/generation)*

Gene substitution	+0·29	
Appearance of deleterious mutant genes	−0·14	0·00
Elimination of the deleterious mutant genes	+0·14	
Total	+0·29	

We are now in a position to estimate the total amount of genetic information which has been accumulated since the beginning of the Cambrian epoch. Prior to this we know very little about the actual forms of life on the earth because of the scarcity of fossil records. Through the following epochs our knowledge of the major course of evolution is fairly good. Before we do this, it may be instructive to see how effectively a high level of improbability in genetic organization can be generated by natural selection. With $H = 0{\cdot}29$ bit per generation, the amount of genetic information accumulated over 1,000 generations is 290 bits. On the other hand, according to Eddington, the total number of electrons in the universe is $\frac{3}{2}.136.2^{256}$, or approximately $2{\cdot}36 \times 10^{79}$. Thus, the probability that a randomly chosen electron out of the universe happens to be the preassigned one is the reciprocal of this number and the corresponding measure of improbability is about 263 bits. This means that 1,000 generations of natural selection can achieve something more improbable than this. But, for the actual process of organic evolution, a duration of 1,000 generations is a very short time indeed.

We do not know how old life on earth is, though there are some reasons to believe that it has existed 2 billion years. We do know, however, that in the Cambrian epoch, which started about 500 million years ago, the earth was already inhabited by organisms such as jellyfish, annelid worms, trilobites, crustaceans, etc.

If we assume then that the genetic information has been gained at the rate of 0·29 bit per generation, the total amount of genetic information accumulated since the beginning of Cambrian epoch is

$$\frac{0{\cdot}29 \times 5 \times 10^8}{\bar{G}} = 1{\cdot}45 \times 10^8/\bar{G},$$

where $\bar{G}$ is the harmonic mean of the duration of one generation in years. Unfortunately, for organisms which do not exist except as fossils, it is impossible to measure the exact length of their generations. All we can do is to infer them from contemporary analogues. For various groups of animals, there is some tendency for smaller and less differentiated members to mature more quickly than the larger and well-differentiated ones. Now, in the history of evolution, it is known that it was always the former type of animal which has succeeded in leaving descendants. Furthermore, $\bar{G}$ is expected to be much smaller than the arithmetic average of the lengths of one generation. With no reliable estimates available at present, I assume, as a biologically reasonable guess, that $\bar{G}$ is of the order of one year.

We may conclude then, that the total amount of genetic information which has been accumulated since the beginning of the Cambrian epoch along the lineage leading to higher mammals may be of the order of one hundred million bits (10^8 bits).

Corresponding to this increase in genetic information, there has occurred a tremendous improvement of phenotypic organization which is implied in the term evolution in the usual sense of the word.

STORAGE AND TRANSFORMATION OF GENETIC INFORMATION

Owing to the recent development of bacterial and viral genetics and also of DNA chemistry, it has become increasingly clear that DNA (deoxyribonucleic acid)

molecules forming chromosomes are the carriers of genetic information. From this standpoint, a chromosome may be considered as a linear sequence of nucleotide pairs, of which four kinds are discriminated.

Muller (1958) estimated the total number of nucleotide pairs which may be present in the chromosome set of man as approximately 4×10^9, by dividing the total mass of DNA contained in a human sperm (*ca.* 4×10^{-12} gr.) by the mass of one nucleotide pair (*ca.* 10^{-21} gr.). Thus, with four kinds of nucleotide pairs, the maximum amount of genetic information that may be stored in the haploid chromosome set of man amounts to

$$\log_2 4^{4\times10^9} = 8 \times 10^9 \text{ bits},$$

and twice as much for the diploid set:

$$1{\cdot}6 \times 10^{10} \text{ bits}.$$

This is the maximum amount of genetic information that may possibly be stored in the nucleus of a fertilized human egg, if the four kinds of nucleotide pairs are equally efficient.

It is generally accepted that the information in DNA is transferred, via RNA (ribonucleic acid) molecules, to proteins, and if, as some workers in this field assume (cf. Crick *et al.*, 1957), a sequence of three nucleotides determines one of twenty possible amino acids, the above value should be reduced by a factor of

$$\log_2 20/\log_2 4^3 \approx 0{\cdot}72,$$

giving

$$1{\cdot}15 \times 10^{10} \text{ bits},$$

or roughly 10^{10} bits as the maximum amount of genetic information that might effectively be stored in the diploid chromosome set.

Here the chromosome set may be compared with an electronic computer. In IBM 650, for example, there are 2,000 memory locations and it can store 20,000 digits, or about $6{\cdot}64 \times 10^4$ bits of information. A more interesting object for comparison is the self-reproducing machine envisaged by von Neumann. According to Kemeny (1955), von Neumann's machine consists of a basic box of 80×400 squares plus a tail containing 150,000 squares. The basic box has a function analogous to the soma, while the tail contains the instructions of the machine and is analogous to the chromosome set. This tail consists of 150,000 cells which are in either an 'on' or 'off' state, and therefore it may store

$$1{\cdot}5 \times 10^5 \text{ bits}$$

of 'genetic information'.

These comparisons not only show the tremendous complexity of chromosome structure, but also reveal an indeed amazing efficacy of DNA codes—efficacy of such an extent, as pointed out originally by Muller (1935), that all of the chromosomes present initially in the fertilized eggs from which the present population of the world (some two thousands of millions) developed would occupy a volume about equal to that of an ordinary aspirin tablet.

Deciphering DNA codes, i.e. learning to read the genetic language is a very fascinating problem which was vigorously attacked for the first time only a few years ago (cf. Yčas, 1958) and, without a Rosetta stone, it would be solved only by statistical treatment, though no success seems to have been obtained so far.

We have estimated, in the previous section, that the total amount of genetic information which has been accumulated since the Cambrian epoch is of the order of 10^8 bits. On the other hand, as shown above, the maximum amount of genetic information that might be stored in the diploid chromosome set of man may be of the order of at least 10^{10} bits. If the first estimate (10^8 bits) is correct, the difference between these two estimates must be real, even if we admit that our Cambrian ancestors had already accumulated a considerable amount of genetic information. If so, I believe that this difference can be interpreted in two ways, namely, either the amount of genetic information which has been accumulated is a small fraction of what can actually be stored in the chromosome set or, more probably, the DNA code itself is highly redundant. In a stimulating paper, to which more attention should be paid by Western geneticists, Schmalhausen (1958), a Russian geneticist, points out that higher reliability of transmitted information may be achieved by the repetition of information, such as repetition of equal genes (polygenes), 'repeats' of gene complexes and in particular diploidy or polyploidy. Furthermore, there may be repetition or a certain kind of redundancy of information within each gene or 'cistron'.

Recently, Sueoka (1960) has made an extensive survey on the guanine–cytosine (G–C) content of deoxyribonucleic acid (DNA) taken from various organisms ranging from bacteria to man. It has been found that for vertebrates the average content lies within the range of 40 to 44%. For various species of bacteria, it varies over a much wider range of $25 \sim 75\%$, indicating the marked divergence in phylogeny. On the other hand, the G–C content of DNA molecules within an organism has a rather narrow distribution with 2σ (twice the standard deviation) of some 6% or usually less around its specific mean value $\overline{p}$. In the case of native DNA taken from the calf thymus, the average G–C content, $\overline{p}$, is about 40% and its heterogeneity, 2σ, is 9·6%, which has been the largest value ever observed.

It may not be difficult to see that if the arrangement of G–C pairs and A–T pairs were entirely at random, the proportion, p, of G–C pairs between molecules within an organism should be distributed unimodally with a binomial variance of

$$\sigma_p^2 = \frac{\overline{p}(1-\overline{p})}{b}, \tag{7}$$

where b stands for the number of base pairs (sum of G–C and A–T pairs) composing a DNA molecule. In the actual case, the number of base pairs per molecule is not constant and we should use its harmonic mean for b. In the case of calf thymus DNA, the harmonic mean is roughly 10^4 and in most cases b should be at least several thousands. Thus, with $b = 10^4$, the expected heterogeneity ($2\sigma_p$) calculated for $\overline{p} = 0{\cdot}3 \sim 0{\cdot}7$ is

$$0{\cdot}009 < 2\sigma_p < 0{\cdot}01,$$

i.e. it lies between 0·9 and 1·0%. If b is half as large, the heterogeneity will become about 1·4 times as large, and even if b is $\frac{1}{4}$ as large, it will only double the above value. On the other hand, the observed heterogeneity is some 6% in vertebrates (Sueoka, 1960), much larger than that expected from the binomial distribution. Thus the ratio between the observed and the expected heterogeneity is roughly 6 in terms of standard deviation and 36 in terms of variance.

I assume that this discrepancy between the observed and the expected is due to repetition in the pattern of base arrangement within the DNA molecule. A simple analysis of our ordinary language will help us greatly to clarify this point. It is known that, in English sentences, the most frequent letter is 'e', and this is followed by 't' or 'a' in frequency. These three letters, together with a space between words, make up about 40% (analogous to G–C pairs in 'genetic language'). I extracted fifty lines, each with seventy letter positions, from a paper on genetics and calculated the mean frequency of 'e', 't', 'a' and space per line and the standard deviation of the frequency between different lines. As shown in the second row of Table 2, the ratio between the observed and the expected standard deviation is about 0·92.* Here the expected standard deviation is calculated from (7) using $\bar{p} = 0{\cdot}439$ and $b = 70$. Similar calculations were performed for lines taken from genetical papers written in French, German and Russian. The results are also listed in Table 2.

Table 2. *Mean and standard deviation of the relative frequency of the sum of 'e', 't', 'a' and space per line in samples of seventy letter positions. The figures in the last column denote the ratio between observed and expected standard deviations. For each language, fifty lines were extracted for calculation.*

Language	Mean (p)	Standard deviation (σ) observed	Standard deviation (σ_p) expected	Ratio
English	0·439	0·0547	0·0593	0·922
French	0·450	0·0539	0·0616	0·875
German	0·381	0·0544	0·0601	0·905
Russian	0·290	0·0464	0·0535	0·867

It may be seen from this table that the ratio between observed and expected heterogeneity in 'e, t, a, space' content per line is roughly 0·9, which is very different from what we have found in the G–C content per DNA molecule.† Suppose we duplicate each letter fifty times so that each line now consists of 50×70 or 3,500 letter positions. By this duplication, the observed heterogeneity does not change but the expected heterogeneity may be reduced to about 1/7·1 because b should be taken as 3500. Then, the ratio between the observed and expected becomes about 6·4, which is similar to the ratio obtained for DNA.

Granting that there may be some thirty-six repetitions in the arrangement of

* This value is mainly due to the negative correlation between neighbouring letters. Giving value 1 for 'e', 't', 'a' and space and value 0 for the remaining letters, I obtained correlation coefficients of −0·20 between two adjacent letters, −0·02 between two neighbouring letters once removed, etc.

† See note at end of paper.

base pairs in DNA molecule, the next question is how such repetitions can be visualized. The analogy with our ordinary language may help us again.

Let us take, as an example, the following sentence:

IT IS SO.

From this sentence we can derive various forms of letter arrangements by duplicating each letter twice. Among them, the following three are especially significant, which I will tentatively call letter, word and sentence repetitions respectively:

(i) Letter repetition: IITT IISS SSOO
(ii) Word repetition: ITIT ISIS SOSO
(iii) Sentence repetition: IT IS SO IT IS SO

These three forms are, as such, indistinguishable with respect to the ratio between the observed and the expected 'heterogeneity'. However, by splitting each of these into pieces of certain length and studying their heterogeneity, we will find that they behave quite differently. Suppose we split each into two pieces of equal length. In the case of letter repetition, the observed variance will become double, because each piece contains only one half of independent letters of the original sentence. On the other hand, in the case of sentence repetition, the variance will remain the same because each piece contains the full sentence. The situation is intermediate in the case of word repetition and the variance becomes 5/3. Returning to the problem of heterogeneity in G–C content, Sueoka (1959) found that by splitting calf thymus DNA molecules by ultrasonic vibration into pieces of about one-tenth in size, the heterogeneity variance increases very little (order of 10% if any). Since we have already assumed that there may be thirty-six repetitions of letters in DNA, this result may be interpreted as showing that repetition in the DNA molecule must be more near to the type of sentence repetition than that of word repetition. Assuming 10^4 base pairs per DNA, then each sentence consists of roughly 300 letters.

The above hypothesis may be tested by splitting DNA molecules into much smaller pieces consisting of less than 300 base pairs and by seeing if the observed and the expected heterogeneity agree with each other.

It should be noted here that the repetition may not be exact in the actual DNA molecule, rather at each repetition the 'word' may be slightly modified from one to the other, like variations in music.

At any rate, through the process of individual development (ontogeny), the genetic information is finally transformed into phenotypic information, with its various aspects in morphology, physiology and behaviour, admittedly a large amount of redundancy being involved among them. Then, how large is the phenotypic information of higher mammals, or specifically that of man? Perhaps the more pertinent question to ask here is how much more phenotypic information is contained in higher animals or man as compared to their Cambrian ancestors. In this sense, the information content should not be counted in terms of atomic or molecular configurations, but should be done in terms of the three-dimensional anatomical structure plus chemical data, as pointed out by Elsasser (1958). He

suggests that, since the information content of human species pertaining to gross anatomy alone could hardly be diagrammed on a plane area of 1 m^2 in which the smallest unit of discrimination is 1 mm^2, and since gross anatomy can only be a moderate fraction of the information content of the organism, the information content of the human organism must be at least of the order of 10^7 bits or, more probably, 10^8 bits. Elsasser states that even a figure of 10^9 bits would hardly appear fantastic. However, since the phenotypic information is transformed genetic information, the former cannot be larger than the latter, which we have estimated as being of the order of 10^8 bits. The correspondence between the genetic and phenotypic information turns out to be quite close considering that, while new genetic information can only be gained through natural selection acting on genotypes, this action is mediated by the phenotypes which are determined by the genotypes. A more reliable estimate will be supplied in the future by anatomists or chemists who will have access to a proper statistical methodology.

In my opinion the creative role of natural selection, which is still not infrequently overlooked by evolutionists, may most convincingly be brought to light by calculating its power of accumulating genetic information and considering the phenotypic complexity as its product. Lerner (1959) states that the meaning of natural selection as a creative process may be well illustrated by quoting Michelangelo's concept of creation: 'The sculptor's hand can only break the spell to free the figures slumbering in the stone.' Indeed, any elaborate work of art must contain a large amount of information.

SUMMARY

1. In the course of evolution, complicated organisms have descended from much simpler ones. Since the instructions to form an organism are contained in the nucleus of its fertilized egg, this means that the genetic constitution has become correspondingly more complex in evolution. If we express this complexity in terms of its improbability, defining the amount of genetic information as the negative logarithm of its probability of occurrence by chance, we may say that genetic information is increased in the course of progressive evolution, guided by natural selection of random mutations.

2. It was demonstrated that the rate of accumulation of genetic information in adaptive evolution is directly proportional to the substitutional load, i.e. the decrease of Darwinian fitness brought about by substituting for one gene its allelic form which is more fitted to a new environment. The rate of accumulation of genetic information is given by

$$H = \frac{L_e}{\log_e 2} \approx 1{\cdot}44 L_e \quad (\text{'bits'/generation}),$$

where L_e is the substitutional load measured in 'Malthusian parameters'.

3. Using $L_e = 0{\cdot}199$, a value obtained from the application of the 'principle of minimum genetic load' (cf. Kimura, 1960*b*), we get

$$H = 0{\cdot}29 \text{ bit/generation}.$$

It was estimated that the total amount of genetic information accumulated since the beginning of the Cambrian epoch (500 million years) may be of the order of 10^8 bits, if evolution has proceeded at the standard rate.

Since the genetic information is transformed into phenotypic information in ontogeny, this figure (10^8 bits) must represent the amount of information which corresponds to the improved organization of higher animals as compared to their ancestors 500 million years back.

4. Problems involved in storage and transformation of genetic information thus acquired were discussed and it was pointed out that the redundancy of information in the form of repetition in linear sequence of nucleotide pairs within a gene may play an important role in the storage of genetic information.

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NOTE ADDED IN PROOF

After this paper had been sent to press, I had the privilege of seeing a preprint of a paper by J. Josse, A. D. Kaiser and A. Kornberg, who successfully determined the nearest neighbour sequence of nucleotides in DNA taken from various organisms. From their Table VI, I calculated the correlation between two adjacent nucleotide pairs in calf thymus DNA, giving value 1 for a G–C pair and 0 for an

A–T pair. The correlation coefficient obtained was about $-0{\cdot}09$, a value not drastically different from the one obtained for English sentences. Similar calculations for bacterial and bacteriophage DNA's (Tables VIII and IX) gave correlation coefficients of at most a few per cent (either positive or negative). Cf. Josse, Kaiser & Kornberg (1960), Enzymatic synthesis of deoxyribonucleic acid. VIII. Frequencies of nearest neighbor base sequences in deoxyribonucleic acid, *Jour. biol. Chem.* (in press).

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Genetical analysis of methionine suppressors in *Coprinus*

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INTRODUCTION

The gene has long been considered as a unit of recombination and also as a physiological unit defined by a complementation test in the double heterozygote. Until some fifteen years ago a gene was thought to be a unit that could have several different alleles which would not complement one another in the double heterozygote and which did not recombine. There was thought to be an absolute correlation between the absence of recombination and absence of complementation. But this is no longer true. Work with the fine analytical techniques now available has shown that low frequencies of recombination are common between alleles that do not complement; but nevertheless low percentages of recombination are still more frequently accompanied by absence of complementation than by its presence. Furthermore, many examples of complementation between some pairs of alleles with extremely low recombination are known in several systems and organisms (Pontecorvo, 1958; Catcheside & Overton, 1958; Giles, 1958; Case & Giles, 1960).

The other well-established correlation, that is between recombination of more than fractional amount and complementation, has so far no true exception. The highest recombination between genes that do not complement in a double heterozygote is 0·5% in *Drosophila pseudoobscura* (Koske & Maynard Smith, 1954), and this is exceptionally high (Pontecorvo, 1958). With higher frequencies of recombination complementation is the rule.

The present study of *Coprinus lagopus* of genes which suppress a mutant requiring methionine has revealed the first real exception to this rule. Two genes 28 units apart do not complement. Thus all the combinations of genetic structure as measured by recombination and physiological function as revealed by complementation are now known.

It would be interesting to consider whether the present example is a rare exception or whether it becomes general and follows the path of previous rare exceptions, such as pseudoalleles and complementation between alleles.

MATERIAL

The cultures were derived originally from fruiting bodies collected in the wild. They were identified as *C. lagopus*. Three wild-type stocks have been used in the experiments, but for the detailed analysis of the suppressors of the *me*-1 locus, two stocks only have been used. Details of these stocks are given in Table 1.

Table 1. *Mating type, methionine loci and origin of the stocks*

		Mating type	Methionine locus	Origin
Wild type	H_5	A_5 B_6	+	Bayford
Methionine mutant	PR2301	A_2 B_3	*me*-1	UV
	PR2205	A_3 B_3	*me*-2	UV
	CR525	A_3 B_3	*me*-3	UV
	P831	A_3 B_3	*me*-4	UV
	G1905	A_6 B_6	*me*-5	UV
	PR7905	A_5 B_5	*me*-6	UV
	M1	A_6 B_6	*me*-7	UV

Life-cycle

Coprinus lagopus has a life-cycle which is fairly typical of its group of fungi, the hymenomycetes, but differs from other groups of fungi (see Fig. 1). The sexual spore produced from meiosis gives rise to a monokaryotic mycelium. This mycelium

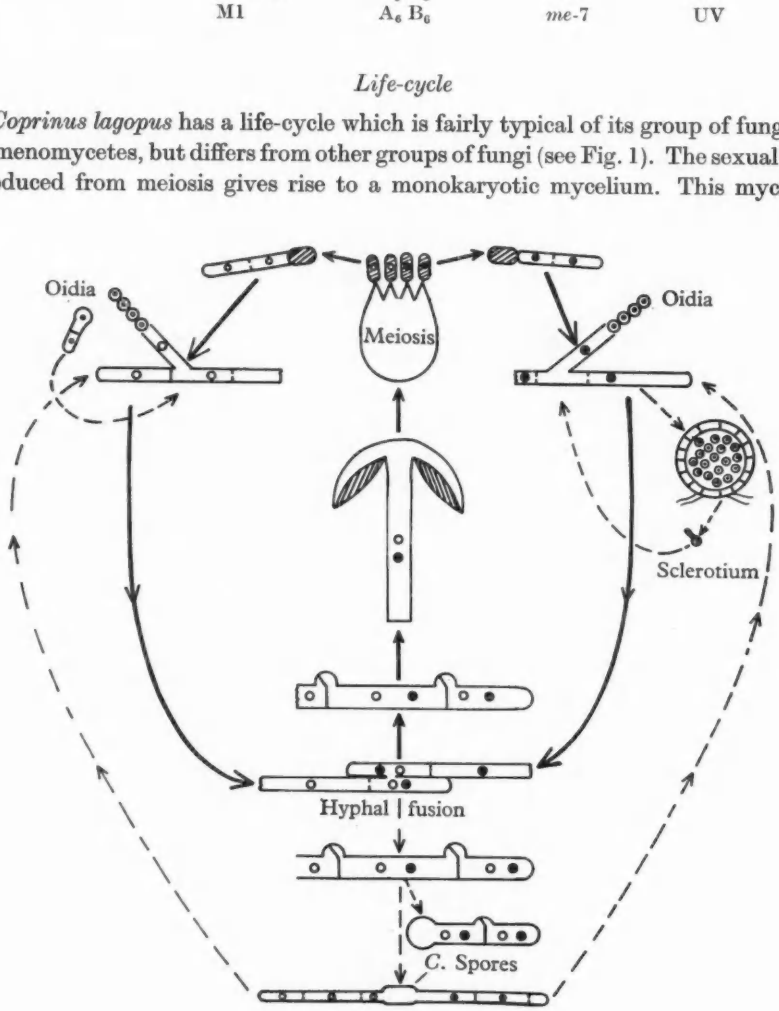


Fig. 1.

produces abundant uninucleate asexual spores, oidia. Pairs of monokaryons of the right mating types fuse by hyphal anastomosis and produce a dikaryotic mycelium which regularly has two nuclei, one from each parent, in each cell. The mycelium of these two types is very distinct, and this is a factor of some importance in the present studies. The monokaryon has thin mycelium, 3 μ in diameter, has normal transverse cross walls, branching which is at a wide angle, 45–80°, produces asexual oidia and rarely if ever produces fruiting bodies. The dikaryon has thick mycelium, 7 μ in diameter, has clamp connexions at the crosswalls which protrude from the hyphae, branching which is acute-angled at about 20°, does not produce asexual oidia, but produces sexual fruiting bodies (see Plate I (*a*) and (*b*)). The growth rate of the dikaryon is about twice as fast as that of the monokaryon. The dikaryon produces large asexual spores, chlamydospores which are somewhat variable in shape and size but which fall into two main classes: (1) round spores which germinate with a single hypha bearing clamp connexions and are dikaryotic, and (2) long spores which germinate with a hypha from each end, not bearing clamp connexions, and are monokaryotic. These spores are useful for resolving the otherwise stable dikaryon into its component monokaryons without the intervention of nuclear fusion and meiosis (see Plate I (*c*) and (*d*)).

Mating is controlled by two incompatibility loci *A* and *B*, each of which has a large multiple allelic series. A compatible mating leading to the establishment of a dikaryon is obtained between cultures which have different alleles at both the *A* and *B* loci. Unstable heterokaryons can be produced when forced by complementary auxotrophic mutants between stocks having the same *B* alleles but differing at the *A* locus. These produce incomplete clamp connexions—'pseudoclamps'—which readily distinguish them from dikaryons.

The fruiting body in which nuclear fusion occurs followed immediately by meiosis, produces basidiospores in unordered tetrads. A generation can take 10 days under favourable conditions.

METHODS

Culture medium and conditions of culture

The minimal medium found to be most satisfactory for *C. lagopus* is L. Fries' medium (Fries 1953). The nitrogen source is ammonium tartrate and asparagine and the only supplement needed is thiamin. Complete medium includes 0·75 g. casein hydrolysate, 0·75 g. yeast extract, 1·13 g. malt extract and 1·5 ml. nucleic acid hydrolysate per litre. For the production of sexual fruiting bodies sterile horse-dung, 25 g. in half-pint milk bottles, is used. *C. lagopus* will fruit on minimal medium, but the fruiting bodies are small and with some stocks they take much longer to develop than on dung. For mycelial growth cultures were incubated at 37° C.; for fruiting, the cultures were initially incubated at 37° C. for 2 days and then at 26° C.

Selection of methionine-independent prototrophs

Uninucleate oidia of the methionine mutant stocks were obtained in large numbers from tube slant cultures, which were 5 days old. The oidia were suspended in water

by pouring sterile water into the tubes and raking the surface of the agar. The suspension was filtered through a loose fibre-glass filter to remove mycelial fragments, and plated onto solid minimal medium at approximately 5×10^6 per plate. Control plates, for obtaining the percentage germination, were made by plating 200 oidia on solid complete medium. After 4 days at 37° C. the plates were counted for colonies, and isolations were made from the minimal plates to tubes of minimal medium.

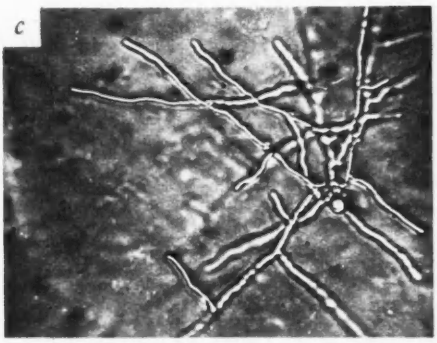
Testing the prototrophs

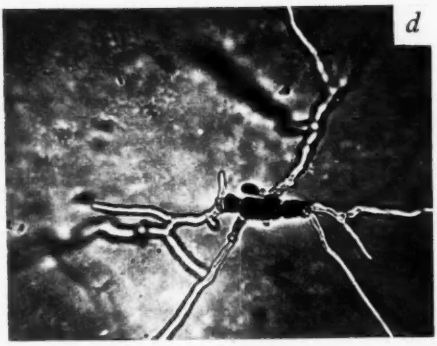
The prototrophs were isolated onto minimal tubes by taking one mycelial transplant from each colony on the minimal plate. The few ungerminated oidia which would be inadvertently transferred were disregarded because they would not germinate in the minimal tubes and would soon be overgrown by the prototrophs. All prototrophs were tested for the type of mutation which had occurred by mating on complete medium each prototroph to a *me*-1 stock with suitable mating-type alleles; for example, stock LR4, $A_5 B_6$ *me*-1. The dikaryon so formed was then tested on minimal medium by transferring a small piece of agar with dikaryotic hyphae. If the dikaryon requires methionine for growth dikaryotic hyphae grow out from the transplant block into the minimal medium 0·2 to 1·0 mm. only. They then stop growing, and if one or both of the components of the dikaryon can grow on minimal medium, then monokaryotic hyphae grow out from the dikaryon. This can be distinguished without any doubt by the absence of clamp connexions and the wide-angled branching and thin hyphae as contrasted to the clamp connexions, and the acute-angled branching of the dikaryon (see Plate I (*e*) and (*f*)).

If the dikaryon does not require methionine, then it will grow at the normal 5 mm. per day. This test for growth on minimal medium gives an immediate discrimination between certain kinds of mutants. Normal growth on minimal medium of the test dikaryon can result from a back-mutation to a wild allele of the *me*-1 gene

Table 2. *Dikaryon test of prototrophs after mating with* me-*1 and testing growth of dikaryon on minimal medium*

Growth on minimal medium	Dikaryon: Known tester nucleus	Dikaryon: Unknown tester nucleus	Conclusions
+	*me*-1 +	+ +	Back mutation to wild allele of *me* locus
+	*me*-1 +	*me*-1 *Sup*	Mutation to dominant suppressor
−	*me*-1 +	*me*-1 *sup*	Mutation to recessive suppressor
−	*me*-1 +	*me*-1′	Back mutation to recessive 'wild' allele at *me* locus





(*a*) Monokaryotic mycelium. × 100
(*b*) Dikaryotic mycelium; arrow points to clamp connexion. × 100
(*c*) Germinating round chlamydospore from dikaryon producing dikaryotic mycelium. × 200
(*d*) Germinating long chlamydospore from dikaryon producing monokaryotic mycelium. × 200
(*e*) Petri dish of minimal medium with eleven transplants of dikaryons—six of the dikaryons are able to grow, five are unable to grow
(*f*) Mycelial growth on minimal medium of dikaryon which will not grow on minimal medium. Left-hand bottom corner, agar block of transplant, arrow points to the point of resolution into the monokaryon—below arrow is dikaryon, above is monokaryon. × 150

(*Facing p.* 144)

or from a mutation to a dominant suppressor. No growth on minimal medium can result from a mutation to a recessive suppressor of the methionine gene or, if such a possibility exists, to a back-mutation of the methionine gene which has restored wild-type phenotype, but is recessive to the mutant allele which requires methionine (see Table 2).

Treatment of oidia with ultra-violet light

Suspensions of oidia in water were irradiated with a Phillips T.U.V. 6-watt mercury vapour lamp mounted in a glass-lined Perspex housing so that the radiation source was 13 cm. from the oidia. The average energy falling on 1 sq. cm. per second at this distance was 64/65 microwatts. The times of irradiation varied from 4 to 6 minutes. Samples of irradiated oidia were plated on complete medium in order to obtain the percentage kill and on minimal medium in order to obtain prototrophs. The percentage kill varied from 96 to 99%.

RESULTS

Characterization of methionine mutants

The seven methionine mutants are all mutants of different loci, which recombine and which mutually complement in dikaryons. Mutants were tested on complete medium, on minimal medium with methionine, and on medium with known precursors in the synthesis of methionine. The results are given in Table 3.

Table 3. *Growth of methionine mutants in mm. in 4 days on minimal, complete and supplemented media*

	Minimal medium	Complete	Methionine	Homo-cysteine	Cystathio-nine	Cysteine	Homo-serine
me-1	—	15	19	8	0	0	2
me-2	—	15	16	0	0	0	0
me-3	—	17	12	8	0	9	0
me-4	—	19	12	0	0	0	0
me-5	—	25	27	3	0	0	0
me-7	—	19	18	9	3	11	0

Spontaneous and induced mutation rate

The detailed analysis has been made with stock PR2301 *me*-1. Using a total of 28×10^6 oidia in two separate experiments prototrophs of two types appeared spontaneously on minimal medium. There were large diffuse colonies and small compact colonies. The germination of the oidia on complete media was 20%. The total number of prototrophs obtained was 64 large diffuse and 96 small compact colonies. These figures represent frequencies of 2·3 per 10^6 plated oidia for the large colonies and 3·4 per 10^6 for the compact small colonies. If allowance is made for the figure of 20% germination, the spontaneous rate is as follows:

Large diffuse colonies—11·5 per 10^6 viable oidia.
Small compact colonies—17·0 per 10^6 viable oidia.

The other six methionine mutants were tested in a similar way for prototrophs. Mutants *me*-2 and *me*-7 gave prototrophs; *me*-3 gave prototrophs after the oidia had developed at 26° C., but not after the oidia had developed at 37° C. The mutant *me*-6 was too leaky to be able to carry out the test, and mutants *me*-4 and *me*-5 did not give prototrophs under any conditions. Ultra-violet light treatment increased the frequency of prototrophs. A dose of u.v. given to PR2301 *me*-1, which resulted in a 98% kill, produced 260 large diffuse colonies and 460 small compact colonies per 10^6 viable oidia. A dose which resulted in a 40% kill produced 6·5 large diffuse and 4·0 small compact colonies per 10^6 plated oidia. Thus even if the killing of 40% of the spores is not taken into account, there is still nearly a threefold absolute increase in the frequency of large diffuse prototrophs due to the u.v. treatment.

Test for recessiveness and nature of the prototrophs

Sixty-six prototrophs obtained on minimal medium from the oidia of the stock PR2301 *me*-1 mating type A_2B_3 were mated to LR4 *me*-1 A_5B_6. The resulting dikaryons were tested for their ability to grow on minimal medium. Eleven of these prototrophs had been obtained after ultra-violet light treatment, but these appeared to be similar to the spontaneous prototrophs and are not considered separately. All of these dikaryons failed to grow on minimal medium, and all, after variable periods of time, resolved into a monokaryon, which would grow on minimal medium. This monokaryon was the prototrophic component. This clear-cut result showed that the *me*-1 prototrophs are not back-mutations to wild-type at the *me*-1 locus and that they are not dominant suppressors. They are either (1) partial mutations at the *me* locus which are wild in phenotype, but are recessive to the full *me*-1 mutant, or (2) are recessive suppressor mutations at another locus (cf. Table 2).

To test further the prototrophs for the types of mutations a sample of twelve of them were mated to a wild-type stock, H_5. The basidiospores were sown on complete medium and the resulting colonies tested on minimal medium. In all cases auxotrophs appeared in varying percentages from a maximum of 25%. This proved that the change was not at the *me*-1 gene, but at some other gene which suppressed the requirement for methionine. The fact that all the prototrophs analysed are recessive suppressors may represent the full spectrum of prototrophic mutations for the *me*-1 locus. The oidia used in the experiments are uninucleate, therefore recessives as well as dominant mutations, if they occurred, would be selected. During the course of the experiments many hundreds of monokaryotic mycelial transplants containing large numbers of nuclei have been placed on minimal medium and none have sectored to produce a prototroph. The cells of the mycelium are uninucleate, and the fact that no prototrophic sectors appear and that recessive suppressor mutations are common in oidia must mean that a multicellular length of the hyphae acts as a biochemical unit as far as the methionine synthesis and its suppressor system are concerned. Only dominant mutations would be expressed and selected in such a multinucleate hyphal transplant, and the fact that they are not found indicates that dominant suppressors of the system are extremely rare or non-existent.

Complementation

From the crosses of the suppressor mutants to wild-type or to LR4 *me*-1, both of which have $A_5 B_6$ mating type, recombinants of the type $A_5 B_6$ *me*-1 suppressor were selected. These were then crossed to all the other sixty-five *me*-1 prototrophic mutants which have $A_2 B_3$ mating type. The resulting dikaryons were tested on minimal medium. Growth of the dikaryon indicates that the suppressors in the two parents do not complement, failure to grow indicates that the suppressors in the two parents complement one another. The effect of complementation or the lack of it on the growth of the dikaryon and the genetical interpretation are given in Table 4.

Table 4. *Genetic interpretation of dikaryons containing two suppressor mutants*

Components of Dikaryon				Growth on minimal medium	Complementation of suppressor
me-1 *sup*-1	+	*me*-1 *sup*-1	+	+	Do not
me-1 + *sup*-2		*me*-1 + *sup*-2		+	Do not
me-1 *sup*-1	+	*me*-1 + *sup*-2		−	Do

On the basis of these complementation tests, the sixty-six prototrophs could be readily classified into three groups. Dikaryons formed between components within each group would, as a rule, grow without methionine and therefore did not show complementation. Dikaryons with components of different groups would, in all cases, not grow without methionine and therefore showed complementation. All pairwise combinatorial tests were not made because of the labour of obtaining the necessary recombination stocks with the right mating type.

The results obtained with group 1 prototrophs are given in Table 5. All but seven of the 392 dikaryons which were tested are able to grow without methionine. There are twenty-eight prototrophs in this group and fourteen of them have been tested with all the others. The seven exceptional dikaryons which do not grow on minimal medium and which show complementation are distributed between five of these thirteen which have been fully tested and involve seven of the remaining fourteen. These exceptions have stood up to repetition and appear to be genuine. Sexual recombination data—referred to later—proves this group of prototrophs to be suppressor mutants of the same locus, *sup*-1.

Group 2 mutants have been tested less extensively than group 1. There are nineteen prototrophs in this group, of which five have been tested with all others. All these pairwise dikaryons are able to grow without methionine except two. All the 296 dikaryons containing a component from each of group 1 and group 2 were unable to grow without methionine and therefore showed complementation. From recombination tests this group of suppressors also appears to belong to a single locus, *sup*-2.

The third group of prototrophs contains fourteen different mutants. Owing to difficulties in this group of obtaining suitable recombinants with the mating-type

Table 5. *Tests for complementation between suppressor mutants within group 1 prototrophs. Dikaryons are made between the original prototrophs and their recombinants with suitable mating type.* + = *growth of dikaryon on minimal medium* = *no complementation between suppressors;* − = *no growth on minimal medium* = *complementation*

Original prototrophs A_2B_3 *me*-1 *sup*	Recombinant prototroph A_5B_6 *me*-1 *sup*																								
	0	1	3	4	10	11	16	19	23	32	34	36	37	39	40	41	42	43	44	45	47	49	54	59	60	61	65
0	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
1	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
2	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
3	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
4	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
10	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
11	+	+	+	+						+				+			+	+	+		+	−		+	+	+	
16	+	+	+	+						+				+			+	+	+		−	+		+	+	+	
19	+	+	+	+						+				+			+	+	+		+	+		+	−	+	
23	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
32	−	+	+	+						+				+			+	+	+		+	+		+	+	+	
34	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
36	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
37	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
39	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
40	+	+	+	+						+				+			+	+	+		+	−		+	+	+	
41	+	+	+	+						+				+			+	+	+		−	+		+	+	+	
42	+	+	+	−						+				+			+	+	+		+	+		+	+	+	
43	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
44	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
45	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
47	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
49	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
54	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
59	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
60	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
61	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
65	+	+	+	+						+				+			+	+	+		+	+		+	+	+	

loci, only three have been tested with all the others in the group. In general these dikaryons have grown without methionine. There were only three examples of full complementation between the suppressors in which the dikaryon would not grow

Table 6. *Complementation tests within group 3 prototrophs which contains* sup-*3,* sup-*4 and* sup-*5 loci.* + = *between 6–10 mm. growth on minimal medium in 2 days;* +− = *4–6 mm. growth on minimal medium in 2 days;* − = *no growth*

Recombinant	Original *me*-1 prototrophs													
	sup-3			*sup*-4			*sup*-5				?			
	29	30	35	14	25	28	8	13	15	33	17	26	27	31
Sup-3 29	+	+	+	+−	+	+−	+	+	+	−	+	+	+	+
Sup-4 25	+	+	+	+−	+	+−	+−	+−	+−	−	+−	+	+	+
Sup-4 28	+−	+	+	+−	+	+	+	+	+	+	−		+	+

on minimal medium. But there were also ten dikaryons which grew rather slower than the normal rate for dikaryons which contain the *me*-1 locus and two non-complementary suppressor loci. The results in this group are given in Table 6.

Sexual recombination tests show that this group is by no means homogeneous. The suppressors represent at least two and probably three loci. All the 326 dikaryons formed between this third group and the other two groups of suppressors do not grow on minimal medium and therefore show complementation.

Recombination tests

Tests of recombination between the methionine suppressor genes have been made on a small scale within the groups and between the groups. Within group 1, five different suppressor mutants have been tested for recombination in three crosses. A total of 468 basidiospores were picked from complete medium and tested on minimal. All grew on minimal medium, showing that no recombinant *me*-1+ type had been produced in this number of spores. It is reasonable to assume from this that the group represents mutants at a single locus, and that a selective technique for picking recombinants, if one could be devised in this system, would reveal low frequencies of recombination as has been found within loci in other organisms.

Tests of recombination between group 1 and group 2 suppressors have given, in all three different crosses tested, approximately 25% of basidiospores which did not grow without methionine. Thus suppressor 1 and suppressor 2 show free recombination and are situated on different chromosomes or at least 50 map units apart. Within group 2 prototrophs no recombinants out of 250 tested have been found.

Other information about the location of these two suppressor loci, suppressor 1 and suppressor 2, has been obtained from crosses of these suppressors to the wild-type stock H_5. In all cases with *me*-1, *sup*-1 and *me*-1, *sup*-2 mutants when crossed to H_5, one-quarter of the basidiospore of the progeny required methionine for growth. The expected genotypes are as follows:

Recombinant	$\begin{bmatrix} me\text{-}1 & + \\ + & sup\text{-}1 \end{bmatrix}$	− on minimal medium
Parental	$\begin{bmatrix} me\text{-}1 & sup\text{-}1 \\ + & + \end{bmatrix}$	all + on minimal medium

With free recombination between *me*-1 and the suppressor locus, a quarter auxotrophs are to be expected. Suppressor 1 and suppressor 2 loci are therefore independent of the *me*-1 locus. By testing the three prototrophic genotypes for their mating-type allele an estimate of recombination between the suppressor locus and the *A* and *B* mating-type loci has been obtained. Suppressor 1 shows $33\% \pm 4\%$ recombination with locus *A*, and suppressor 2 shows free recombination with both *A* and *B* loci.

Recombination tests within the third group of suppressors have given varied results (see Table 7).

From the table it seems probable that three different suppressor loci are included in this group. These may be tentatively designated suppressor 3, which comprises

Table 7. *Crosses between suppressor mutants in the third group; both parents have* me-*1 and a suppressor gene. The percentage recombination between the suppressor mutants is obtained by doubling the percentage of auxotrophs*

Parent of cross	Basidiospores on minimal medium		% recombination
	Total	−	
LR17 (Ms 29 recombinant) × Ms 25	286	41	28
× Ms 28	61	7	23
× Ms 14	86	9	21
× Ms 33	73	20	Free
× Ms 30	97	0	—
× Ms 35	84	0	—
LR57 (Ms 28 recombinant) × Ms 33	30	5	Free

mutants Ms 29, Ms 30 and Ms 35, suppressor 4, which comprises Ms 25, Ms 28 and Ms 14, and suppressor 5, which has Ms 33.

Tests of recombination of these suppressor loci in this third group and the methionine *me*-1 locus are given from crosses to wild-type in Table 8.

Table 8. *Recombination percentage between* me-*1 and the suppressor loci from crosses between Ms stock* me-*1*, sup *and* H_5 *wild-type* + +

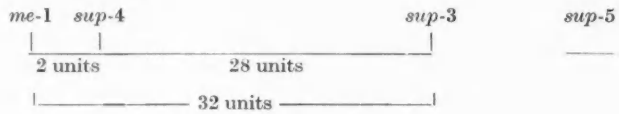
	Total	− on minimal medium	% recombination
Ms 29 × H_5	55	8	29·2
Ms 35 × H_5	100	16	32
Ms 25 × H_5	200	2	2
Ms 33 × H_5	96	28	Free
Ms 15 × H_5	96	26	Free
Ms 8 × H_5	100	29	Free
Ms 13 × H_5	96	27	Free

Again three loci are distinguished: suppressor 3 with about 30% recombination with *me*-1, suppressor 4 with about 2% recombination, and suppressor 5 with free recombination with *me*-1. The two methods of distinguishing the suppressor loci agree completely (see Table 9).

Table 9. *Comparison of the grouping of the Ms prototrophs into three suppressor loci on two different recombination tests*

On the basis of recombination between suppressors			On the basis of *me*-1 *sup* recombination		
sup-3	*sup*-4	*sup*-5	*sup*-3	*sup*-4	*sup*-5
Ms 29	Ms 25	Ms 33	Ms 29	Ms 25	Ms 33
Ms 30	Ms 28		Ms 35		Ms 15
Ms 35	Ms 14				Ms 8
					Ms 13

The linkage between *me*-1 and suppressors 3 and 4 is approximately additive and the location of these three suppressor genes can be tentatively summarized as follows:



The question arises that the different recombination values between the different suppressor mutants and between the suppressors and *me*-1 may be due to chromosomal inversions involving one of the suppressor loci. The pairwise complementation tests within this third group show, in all but three exceptional cases, no complementation (see Table 6). This group on the recombinational data contains three well-separated suppressor loci. If the recombinational data are interpreted correctly, then these suppressors show the hitherto unique property of being independent or at most 28 units apart and yet the double heterozygote does not exhibit complementation. Because of this unique situation, and because the recombination data are based on the identification of one of the recombinant classes, detailed tests were concentrated upon one of the dikaryons, Ms 25 × LR17 (Ms 29 recombinant). On the two-suppressor-loci interpretation these components have the genotypes A_2B_3 *me*-1 *sup*-4 + and A_5B_6 *me*-1 + *sup*-3. Two of the auxotrophic progeny obtained from this cross were crossed to a stock which was *me*-1 *sup*-1 and a wild-type stock + + +. In both cases a 1:1 ratio of prototrophs to auxotrophs was obtained in the progeny. This confirmed completely the identification of the auxotroph as *me*-1 + +. The other three presumed classes, two parental *me*-1 *sup*-4 +, *me*-1 + *sup*-3 and the recombinant class *me*-1 *sup*-4 *sup*-3, were identified by backcrossing the prototrophic progeny to the two parents. The progeny were then tested on minimal for auxotrophs. The two parental types should give no auxotrophs with one parent and the 14% auxotrophs with the other parent. The recombinant with both suppressor loci should give no auxotrophs with both parents. Out of seven tested, the following were obtained:

1 recombinant	*me*-1 *sup*-3 *sup*-4
2 parental	*me*-1 + *sup*-3
4 parental	*me*-1 *sup*-4 +

As we expect equal proportions of the parental types and 14% of the recombinant, this limited but conclusive result proves the separation of these two loci with normal recombination between them. The 2% of recombinants between *me*-1 and *sup*-4 estimated on the production of auxotrophs (see Table 8) was also tested further. The prototrophs in the family, wild-type + + × *me*-1 *sup*-4, were again crossed onto *me*-1 + *sup*-1, and the dikaryons tested on minimal medium. If the 2% of recombinants is accurate, then the prototrophs should have contained 49% of + + genotype and 49% of *me*-1 suppressor genotype, instead of 25% of each and 25%

of + *sup*-4. The reactions on minimal medium of these genotypes in the dikaryon with *me*-1 *sup*-1 is given in Table 10.

Table 10. *The reaction on minimal medium of three prototrophic genotypes in a dikaryon with* me-1 sup-1

Prototrophs	Tester	Expected on independence	Expected on 2% recombination	Growth on minimal medium	Numbers observed
+ + +	*me*-1 + *sup*-1	25%	49·5%	+	50
+ *sup*-4 +	*me*-1 + *sup*-1	25%	1%	+	
me-1 *sup*-4 +	*me*-1 + *sup*-1	25%	49·5%	−	41

The results are in good agreement with the expected on 2% recombination, but differ significantly from the expected on free recombination, $\chi^2 = 5{\cdot}54$, $p = > 0{\cdot}02$. The complete agreement of recombination values rules out the possibility of a chromosome rearrangement being involved.

Suppressor genes and fertility

Coprinus lagopus is similar to *Neurospora crassa* and *Aspergillus nidulans* in being unable to produce fruit-bodies when the dikaryon is homokaryotic for most biochemically deficient mutants despite the fact that adequate supplies of the metabolite are present in the medium. This is true in *Coprinus* for adenine, methionine, choline and nicotinamide mutants. In fact it is true for all the biochemical mutants that have so far been tested on *Coprinus*, with the exception of those that are homokaryotic for para-aminobenzoic acid mutants. Of all the seven methionine loci in *Coprinus* no fruiting has been obtained in homokaryons. If, however, a methionine suppressor gene is present either in a double or a single dose, then they are able to fruit. The effect of these suppressor genes on the ability to produce fruiting bodies is shown below:

Components of dikaryon	Growth on minimal medium	Fruiting
me-1 + + *me*-1 + +	−	No fruiting
me-1 *sup*-1 + *me*-1 *sup*-1 +	+	Fruiting
me-1 + + *me*-1 + *sup*-2	−	Fruiting
me-1 *sup*-1 + *me*-1 + *sup*-2	−	Fruiting

It is perhaps not surprising that the dikaryons which carry suppressor genes which grow on minimal-medium fruit, but the fact that a dikaryon containing one recessive suppressor or complementary suppressors (in which the dikaryon is unable to grow on minimal) also fruits is somewhat surprising. Clearly the suppressor loci are all completely recessive with regard to their effect on the synthesis of methionine and growth on minimal medium, but this is not so in regard to their effect in allowing fruiting to occur. In this respect there is some dominance over the wild-type allele.

DISCUSSION

The present genetic analysis in *Coprinus* shows that at least four and probably five loci, which are widely spaced on the chromosomes, can have recessive alleles that suppress the effects of the methionine mutant *me*-1. How these different suppressor loci and their alleles interact genetically to affect the expression of *me*-1 mutant is known in detail, but what these suppressors are doing biochemically is entirely unknown. There may be a parallel with *Neurospora* in which a suppressor gene acts upon an *me* mutant blocking the cleavage of cystathionine to homocysteine and also on another *me* mutant which blocks an earlier step in the synthesis, the coupling of cysteine and homoserine to form cystathionine (Giles, 1951). The fact that the *sup*-1 in *Coprinus* suppress *me*-1 blocked between cystathionine and homocysteine but not *me*-7 blocked before cystathionine suggests a difference. In *Neurospora* the two blocks in methionine synthesis by the mutants are known to lack the respective enzymes cystathionase 1 and cystathionase 2 (Fischer, 1957). The suppressor restores the production of both enzymes in reduced amount. In general little is known biochemically about the mode of action of suppressor genes (cf. Demerec & Hartman, 1959). There have been suggested two basically different mechanisms: (1) a direct effect on the enzyme restoring its production or a direct repair of some deficiency in the co-enzyme, or (2) an indirect effect removing an inhibition, opening up an alternative pathway. The best-known example of restoration of the enzyme by a suppressor is in tryptophane synthetase in *Neurospora* in which the suppressors are highly specific to certain mutant alleles of the *ts*-locus. But even here the biochemical action appears to be the indirect one of the removal of excess zinc to which the 'mutant' enzyme is excessively sensitive (Suskind & Kurek, 1959). The difficulty of inferring that suppressors which show specificity to certain alleles of the restored gene are restoring the enzyme directly is also brought out by the present study. In the methionine suppressor system in *Coprinus* there is a whole range of action, from non-specific action as shown by the five different suppressor loci which all act upon the same enzyme block, to the highly specific interaction of the suppressor loci and alleles as shown by complementation patterns.

These patterns of complementation between the suppressors have all the usual features well known in other analyses of complementation between pairs of alleles of the same locus (Catcheside & Overton, 1958; Case & Giles, 1960). The complementation pattern found within *sup*-1 locus (see Table 5), as pointed out by my colleague Mr D. H. Morgan, does not, as the data stand, lend itself to a linear arrangement as found in several loci in *Neurospora*. The data in the table on this point, however, are so incomplete that this does not warrant comment without the support of much more detailed analysis of *sup*-1 mutants.

The unique feature about the complementation patterns in the methionine suppressors are the examples of non-complementation between loci that are, in one case, 28 units apart, and in another case, independent. The full explanation of this lack of complementation between distant loci will have to await the biochemical analysis of the system, but certain features about it will be discussed.

The complementation tests are made in a dikaryon so that the lack of complementation between *sup*-3 and *sup*-4 in the double heterozygote is an effect between nuclei and in the cytoplasm. In a haploid nucleus in a monokaryon with wild-type alleles at both suppressor loci there is complementation in the sense that the suppressor system expresses the wild-type phenotype. Of great interest would be the analysis of a diploid or disomic nucleus of the double heterozygote. Would there be complementation under these conditions? Such an analysis has not been feasible in *Coprinus*, but it could be done if a similar case of non-complementation were found in *Aspergillus* or *Neurospora*.

Such a comparison of intra-nuclear and inter-nuclear complementation is relevant from two points of view. Firstly it may help in determining the level of action of the complementation, whether at the RNA template, protein synthesis or at some higher level of dynamic interaction within the cell. Secondly, it may help to explain why such non-complementation between distant loci has not been found before. In other fungi where complementation tests have been made on a large scale in heterokaryons the analysis is based upon a test for complementation between nuclei rather than within a diploid nucleus, except in *Aspergillus* where both intra- and inter-nuclear tests can be made. In *Neurospora* the pseudowild test of Case & Giles (1960) is essentially an intra-nuclear test. In *Aspergillus* and *Neurospora* the intra- and inter-nuclear tests agree, although Case & Giles have reported slight discrepancies between them in the *pan*-2 system.

But so far these analyses in *Neurospora* and *Aspergillus* have been on mutants blocking enzyme action and have not dealt with suppressor genes. It may be that suppressor genes are acting at a different level in synthesis and that such long-distance effects found in *Coprinus* are confined to such genes. As Pontecorvo (1958) suggests, there may be genes which are not directly concerned with protein synthesis and these suppressors may be of this type. The extensive analysis of gene interaction in *Drosophila* has undoubtedly included all types of gene action, and it is tempting to think that the lack of long-distance non-complementation in *Drosophila* is due to the strictly intra-nuclear analysis. But of course a special search may reveal examples. Clearly these suggestions can only be refuted or supported when more examples of long distance non-complementation have been found, and when a close comparison between intra- and inter-nuclear effects are analysed. Perhaps the most likely place to look is in other suppressor systems.

SUMMARY

Seven different genes, *me*-1 to *me*-7, controlling the steps in the synthesis of methionine in the Basidiomycete *Coprinus lagopus* have been tested for the production of prototrophs on minimal medium. Cultures carrying *me*-1 and *me*-7 produced prototrophs spontaneously at a rate of $2{\cdot}6 \times 10^{-5}$. These prototrophs were the result of mutations of suppressor genes and not due to back-mutation of the *me*-gene.

An intensive study of sixty-four mutants of an independent origin suppressing *me*-1 has revealed five different suppressor loci.

Tests for complementation between suppressor mutants and for their recessiveness to the wild allele were made in dikaryons.

All suppressor mutants were recessive to the wild allele. The five suppressor loci were all separated from one another by recombination, twenty-eight map units being the smallest distance between any two pairs. Mutants of the same locus did not complement one another, with few exceptions. Mutants of different loci, as tested in the trans-position in a dikaryon, complemented one another with the exception of pairs between *sup*-3, *sup*-4 and *sup*-5. *Sup*-3 and *sup*-4 are 28 units apart and are independent of *sup*-5 and yet they did not complement. This unique example of long-distance non-complementation is discussed in terms of gene action.

I wish to thank Mr D. Walls for his valuable technical help and Mr D. H. Morgan for help in several ways and in particular for reading the manuscript. The basis of the biochemical genetic analysis in *Coprinus lagopus* was made while the author held a Rockefeller Fellowship at the California Institute of Technology, and I wish to thank the Rockefeller Foundation for facilities and Professor Beadle and Professor N. Horowitz for much helpful discussion.

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SHORT NOTES

XXY MICE

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Welshons & Russell (1959) have presented data to show that the XO chromosomal constitution in the mouse is female. This conclusion was based on results of genetical tests with sex-linked markers and on chromosome counts. All XO females were matroclinous, that is, they had inherited their X-chromosome from their mother. Females of this type will arise when non-disjunction occurs in the meiotic divisions of the father and results in spermatozoa without a sex-chromosome. Alternatively, the paternal sex-chromosome may be lost from the fertilized ovum if non-disjunction of sister-chromatids occurs during the first cleavage division. This latter explanation has been urged by Ohno, Kaplan & Kinosita (1959), who found no evidence for non-disjunction of the X- and Y-chromosome in an extensive cytological examination of the mouse testis.

While the occurrence of matroclinous XO females does not provide definitive proof for non-disjunction in the male, such proof can be furnished by the occurrence of XXY animals with one paternal X-chromosome, for these must have arisen through fertilization with XY-spermatozoa. Welshons & Russell (1959) failed to obtain this type of animal, but postulated that it would be male or intersex if it occurs at all. This is in keeping with the situation in man, where males exhibiting Klinefelter's syndrome have been shown to possess two X's and a Y (Jacobs & Strong, 1959; Ford *et al.*, 1959).

A presumptive case of an XXY male mouse was found by McLaren (1960) in crosses with marked X-chromosomes. Unfortunately this animal died at an early age before it could be tested for fertility or examined cytologically. The present note is a report on two sterile males which on genetical and cytological grounds were shown to be XXY.

As a marker of the X-chromosome a rearrangement causing a variegated-type position effect was employed, the origin and inheritance of which will be described in a separate paper. The males which sired these exceptional animals carried a piece of autosome (linkage group I), with the wild-type alleles of pink-eye and chinchilla, attached to, or inserted into, their X-chromosome. In addition they either possessed two complete autosomes, bearing the recessive genes pink-eye and chinchilla, or albino, or only one complete and one deficient for the piece transposed to the X. In females of the equivalent constitution, the heterozygous transposition causes a variegation for the recessive genes; the transposition and the phenotype it produces have been denoted flecked. Males with the transposition do not show the variegation and are wild type. When such flecked-bearing males are crossed with pink-eye, chinchilla, or albino females all the daughters are flecked, while the sons that do not receive the father's X are of the recessive type.

Among 810 offspring of such crosses there appeared two phenotypically flecked males, both of which were sterile. Since very large numbers of flecked-bearing males had been shown not to show the variegation, the exceptional animals were presumed to be XXY in sex-chromosome constitution, having received from their father both the Y and the X bearing the flecked transposition. They copulated readily with females, but produced no offspring. Cytological studies of the extremely small testes showed the presence of tubules but no spermatogenic cells. Chromosome counts in cells of the corneal epithelium

by the method of Fechheimer (1960) and of the spleen by a modification of the method of Ford & Hamerton (1956) showed that both males had 41 chromosomes.

In the same crosses, there appeared five non-flecked females, i.e. recessive type, which were presumed to lack the paternal X-chromosome. Two of them have been examined for chromosome number so far; both had only 39 chromosomes. It is reasonable to assume that some, or all, of these females arose from eggs fertilized by O-bearing sperm in crosses in which the complementary type XY-bearing sperm has been shown to occur. The XO-constitution of some of these females has been confirmed in crosses with males bearing the sex-linked gene, Tabby (*Ta*), when hemizygous *Ta* daughters were produced.

A point of interest is that the variegated-type position effect is expressed in XX and XXY animals but not in XY ones. This indicates that its expression does not depend on sex or the absence of the Y, but requires the presence of two X-chromosomes. This complements the finding of Russell & Bangham (1960) that XO females do not show position-effect variegation.

Two more presumptive XO females arose in crosses designed to test for linkage between *Ta* and the flecked-transposition. Both were matroclinous in regard to their X-chromosome and may have arisen by non-disjunction in the father. A third XO was patroclinous, for it was phenotypically hemizygous *Ta* and arose in a cross of a *Ta* male with a wild-type female. Genetical tests indicated that it possessed only one X-chromosome. McLaren (1960) has described a similar case, but Welshons & Russell (1959) found none in 275 females which might have included it. In the last two cases it is possible that the mothers of the XO females were themselves XO types, so that the exceptional daughters were produced by normal segregation. This could also explain the appearance of a homozygous-type Tabby female observed by Falconer (1953) in a similar cross. In the present case the exceptional female was one of eighteen daughters. This suggests that the mother was not an XO type, for a 1 : 1 ratio of exceptional to normal daughters would be expected from normal segregation. However, as the XO class appears to be relatively inviable (Welshons & Russell, 1959), there is still a possibility that the mother was an XO type. So far, therefore, there is no clear genetical evidence for non-disjunction in the female mouse.

The overall frequency of non-disjunction in flecked-bearing males was almost 1%, and the few data from the *Ta* crosses suggested a similar frequency of non-disjunction. If the XO females are taken as evidence of non-disjunction in the male, it would appear that this occurrence is no less frequent in normal males than in flecked-bearing males.

A striking feature of the combined data for all males is that, of the nine exceptional offspring (omitting the possible patroclinous female), all but one arose in first or second litters, when the males siring them were less than $4\frac{1}{2}$ months old. The other was found in a litter that had been sired by a 23-week-old male. In addition, the frequency of exceptional offspring decreased from litters sired between weeks 6 and 12 to litters sired between weeks 12 and 18. As yet the numbers are too small to make these observations more than suggestive. It would be of interest if the age of the male were found to influence the frequency of non-disjunction, and this is now being investigated.

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Modifying the sternopleural hair pattern in *Drosophila* by selection

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Two apparently very similar quantitative characters, the numbers of hairs on the sternopleural region and on the abdominal sternites of *Drosophila melanogaster*, show unexpected differences in their genetic behaviour. In particular, the amount of left-right asymmetry of the sternopleurals (i.e. the mean absolute difference in numbers of hairs on the two sides of the fly) tends to decline when inbred lines are intercrossed, and can be both increased and decreased by straightforward selection; the corresponding index for the sternite hairs—the uncorrelated variance between two sternites, or the mean absolute difference between the numbers of hairs on each—appears, on the other hand, to be susceptible neither to selection nor to change when inbred lines are crossed (Mather, 1953; Reeve & Robertson, 1954; Reeve, 1959).

In discussing these differences, Reeve (1959) suggested that they might partly be explained by anatomical differences between the two characters, in that the region covered by the sternite hairs is strictly delimited by the extent of the sternites which they cover, while the sternopleural hairs cover a much more indefinite region with no obvious boundaries. The pattern of the sternopleurals is also less restricted than that of the sternite hairs, which give a fairly even coverage of a definite sclerotized area. Variation in sternopleural asymmetry might thus result from variation in the precision with which the sternopleural region is defined, or in the precision with which the general pattern of the sternopleural hairs is determined, two factors which do not seem likely to affect the sternite hairs.

In order to throw some light on this problem, a selection experiment has been carried out to test how far the sternopleural hair pattern is modifiable genetically without the intervention of major mutations. Typically the sternopleural hairs form a variable dorso-ventral row, with two much larger and more stable bristles, one at each side near its dorsal end, and additional hairs occurring sporadically near them. An imaginary straight line drawn through the bases of the two large bristles divides the hairs into

two groups, a dorsal (D) with usually 1–2 hairs and a ventral (V) with some 6–7 hairs. Selection was carried out to reduce the number ($V-D$) and make it negative if possible, thus piling up hairs on the dorsal side of the line and reducing the extent of the ventral region. This seemed to be the simplest way of altering the hair pattern.

The results of an experiment with the Pacific wild stock, selecting the most extreme 20–25 parents of each sex out of 60–70, and mass-mating them each generation, are shown in Table 1. Here V and D are the sums of the numbers of ventral and dorsal hairs on the two sides of the fly, while left-right asymmetry is measured in the usual way as the mean difference in the number of hairs on the two sides, regardless of sign.

Table 1. *Effects of selection for sternopleural pattern*

Stage of selection	Sternopleural indices		
	$V-D$	$V+D$	$\|L-R\|$
Generation 0	9·00	15·20	1·14
23–26	1·33	16·43	1·21
% change	−85	+8·1	+6·1

There is no doubt that the sternopleural hair pattern is genetically modifiable, since the excess of ventral hairs has been reduced during twenty-three generations of selection from 9·0 to 1·3, or by 85%, and individuals with 1–3 more dorsal than ventral hairs now turn up quite frequently in the selected population.

The changes in the other indices shown in the table are of equal interest. Since V is initially much larger than D, one would expect that selection to reduce ($V-D$) would reduce the total number of hairs ($V+D$). This actually occurred for the first few generations, but since then ($V+D$) has gradually risen until it is now some 8% larger than its initial value. This clearly shows that we have brought about a genuine change in the distribution of the hairs, and have not simply cut off the tail of the ventral group by reducing the region within which they can be produced. Some effect of this kind has probably also occurred. During the course of the experiment no obvious change has been noticed in the size or position of the two large bristles, and it is clear from inspection of the flies that our results have not been produced by moving the two large bristles ventrally.

Mean asymmetry, |L−R|, has only risen by about 6% during the experiment, a change which must be attributed entirely to scale effects, since the asymmetry and the total hair count have risen in almost exactly the same proportion. Now twenty generations of artificial selection with restricted population size must have brought about a marked deterioration in the adapted gene-pool of the population, with a consequent reduction in the general level of developmental homeostasis; but this clearly has not been reflected in any increase in the level of asymmetry. Our experimental results do not, therefore, support the theory put forward by Mather (1953) and Thoday (1958) that asymmetry is a measure of developmental homeostasis. This theory has been criticized by Reeve (1959) on the basis of other experimental data.

Our experiment has demonstrated clearly that the sternopleural hair pattern can be quite strikingly modified genetically, but without changing the level of asymmetry. It does not, therefore, explain the difference in genetic behaviour between the two quantitative characters based on hair count. It seems likely, however, that crossing inbred lines, or selecting directly for asymmetry, brings into play other genetic differences which do actually alter the degree of precision with which the sternopleural hair pattern is determined, and so affect asymmetry. Evidently the whole problem of the genetic control of sternopleural hair number, pattern and asymmetry is more complex than might be supposed, and needs further analysis.

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